The impact of myostatin variants in the South African Bonsmara and Drakensberger beef cattle breeds

By

Rendani Asnath Madula

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Supervisor: Prof Carina Visser

Co-Supervisor: Prof Este Van Marle-Köster

Declaration

I, Rendani Asnath Madula hereby declare that this thesis, submitted for the MSc (Agric) Animal Science: Animal Breeding and Genetics degree at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any other University.

Rendani Madula

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“Meaning and purpose come not from accomplishing great things in the world, but simply from loving those who are right in front of you, doing all you can with what you have, in the time you have, in the place where you are”.

-Katrina Kenison

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Abstract

The Myostatin (*MSTN*) gene has been linked to the double muscling phenomenon, in which a succession of mutations renders the gene inactive and unable to regulate muscle fibre deposition effectively. This study aimed to assess impact of the *MSTN* variants and their combinations, in the South African (SA) Bonsmara and Drakensberger breeds, on reproduction (age at first calving (AFC), inter-calving period (ICP), scrotal circumference (SC), longevity), growth traits (direct birth weight (BW_{DIR}), direct weaning weight (WW_{DIR}), average daily gain (ADG), feed conversion ratio (FCR) and carcass traits based on real-time ultrasound (RTU) measurements (fat, marbling, eye muscle area (EMA)). Genomically enhanced estimated breeding value (GEBVs) and *MTSN* genotypes for SA Bonsmara and Drakensberger animals were available. Thirteen *MSTN* variants were genotyped using the IDBv3 SNP array. This study was divided into a phase 1 and phase 2. In Phase 1, three *MSTN* variants (Nt821, F94L and Q204X) were observed in 355 animals genotyped across the Bonsmara, Beefmaster, Brangus, Drakensberger and Limousin breeds and genotypic and allelic frequencies were estimated. In the Limousin population, the F94L variant was fixated. The Nt821 and Q204X variants were observed at frequencies ranging from 0.00% to 46.00% in the remaining breeds. In the association study (phase 2), only Bonsmara and Drakensberger genotypes were included. Genotypic frequencies of *MSTN* variants ranged from 1.18% for Q204X to 35.02% for Nt748 in homozygous affected animals in Bonsmara cattle and 2.32% for Nt821 to 12.01% for Nt748 in Drakensberger cattle. Association analysis indicated no significant association ($p > 0.05$) between the different variants and reproduction traits (AFC, ICP and longevity), FCR or fat in the Drakensberger population. All *MSTN* variants in the Bonsmara population were significantly associated with longevity ($p < 0.05$). In addition, the Q204X variant was significantly associated with all reproduction and growth traits in the Bonsmara population ($p < 0.05$). The results of the combined genotypes indicated that there was an additive effect when more than one *MSTN* variant was present. This is the first study to report the impact of *MSTN* variants on reproduction, growth, and RTU measurements in SA Bonsmara and Drakensberger breeds.

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List of abbreviations

Aa	Amino acid
ActRIIA/ActRIIB	Activin type two receptors
ADG	Average daily gain
AFC	Age at first calving
Akt	Protein Kinase B
ALK	Activin type one receptors
ANOVA	Analysis of variance
ARC	Agricultural Research Council
BGP	Beef Genomics Program
BLUP	Best Linear Unbiased Prediction
BW_{DIR}	Direct birth weight
BTA	Bovine autosomes
CDI	Cyclin-dependent kinase inhibitor
CDK	Cyclin-dependent kinase
Cdk2	Cyclin-dependent kinase 2
CM	Centimetre
DAFF	Department of Agriculture, Forestry and Fisheries
DNA	Deoxyribose nucleic acid
DnActRIIB	Dominant negative mutant of ActRIIB
E2F	Transcription factor
EBV	Estimate breeding value
EMA	Eye muscle area
FA	Fatty Acid
FCR	Feed conversion ratio
GASP-1	growth and differentiation factor-associated serum protein 1
GDF-8	Growth and differentiation factor 8
GDP	Gross Domestic Products
GEBVs	Genomic estimate breeding values
GLM	General Linear Models
GS	Genomic selection
GWAS	Genome-wide association studies
h^2	Heritability

HWE	Hardy-Weinberg equilibrium
ICP	Inter-calving period
IDB	International dairy beef
KDA	Kilodalton
KG	Kilogram
LRF	Livestock registering federation
LSD	Least Significant Difference
MAF	Minor allele frequency
MAS	Marker assisted selection
MANOVA	Multivariate analysis of variance
Mh	Muscular hypertrophy
MLC	Myosin light chains
MM	Millimetre
MRF	Myogenic regulatory factors
MSTN	Myostatin
MyoD	Myogenic differentiation
p21	Cyclin-dependent kinase inhibitor
PCR	Polymerase chain reaction
QC	Quality control
QTL	Quantitative trait loci
Rb	Retinoblastoma
RFLP	Restricted fragment length polymorphism
ROH	Runs of homozygosity
RTU	Real-time ultrasound
SC	Scrotal circumference
SE	Standard error
Smad	Small mothers against decapentaplegic
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for Social Sciences
SSP	sequence specific primers
TGF-β	Transforming and growth factor-beta
TIA	Technology Innovation Agency
WW_{DIR}	Direct weaning weight

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Chapter 1 Introduction

1.1 Introduction

Agriculture is considered an important sector in the economy of South Africa in terms of employment, contribution to the GDP and foreign exchange earnings (BFAP, 2020). In the South African agricultural sector, the beef industry is the second fastest growing sector, following the broiler sector (DAFF, 2018). Livestock production contributed approximately R142 964 million (50.6%) to the GDP in 2017/2018 (DAFF, 2018). Additionally, the industry contributes significantly to food security by providing animal derived protein much needed by the South African growing population.

The challenges accompanying the fast growing human population to natural resources and especially to food security has been well documented (Visser *et al.*, 2020). Currently there are 60.6 million people in South Africa and this number is expected to reach 75.5 million people by 2050 (StatsSA, 2022). Following this same trend, the demand for red meat is also expected to increase, resulting in an extra burden on livestock producers (Visser *et al.*, 2020). In order to satisfy this growing demand, farmers will be expected to increase the production and efficiency of livestock. To double the production by 2050, livestock producers need to make a concerted effort to increase productivity across all production systems (extensive, intensive, communal, and commercial) to meet the demand for animal products within the country (Garnett *et al.*, 2013; Webb, 2013; Dawkins, 2017).

There are about 14 million heads of cattle in South Africa (SA), consisting of approximately 80% beef cattle, of which 60% are found in commercial production system and 40% in communal production systems (StatsSA, 2022). Beef cattle farming in SA is predominantly extensive where cattle are bred, raised and weaned on natural pastures (Webb and Erasmus, 2013; Grobler *et al.*, 2019). Approximately 70% of weaned calves are fattened and finished in feedlots before slaughter at target weights of 400-450kg (Webb, 2013). This is done to reduce stocking rates in grazing systems, to improve grazing system management and to obtain desired carcass weights (220-250kg) (Webb and Erasmus, 2013).

Approximately 30 cattle breeds are registered in SA including Sanga, *Bos taurus* (European) and, *Bos indicus* (Zebu). The SA Bonsmara is a composite breed of 3/8 exotic (Milk Shorthorn, Hereford) and 5/8 Afrikaner cattle, categorized as a Sanga type (Bonsma, 1980). This breed was developed through a scientific crossbreeding program, supported by performance testing. The SA Bonsmara breed had 120 000 registered cattle, accounting for 41.7% of all registered cattle participating in Logix Beef in 2019 (SA Stud Book, 2019). The Bonsmara breeders' association has enforced visual evaluation for selection for functional efficiency and cattle has been subjected to selection for economically important traits (Bonsma, 1980). In South Africa, the SA Bonsmara was the first beef breed to use genomically enhanced estimated breeding values (GEBVs) (GEBVs; van der Westhuizen *et al.*, 2017), which are predicted using single-step genomic

best linear unbiased prediction (ssGBLUP; Legarra *et al.*, 2014). The GEBVs are an additional tool to assist seed stock breeders to make selection decisions (Meuwissen *et al.*, 2001).

The Drakensberger is an indigenous South African breed which was bred and developed in South Africa over hundreds of years through natural and artificial selection (Scholtz, 2010). One of the breed's most essential characteristics is its ability to adapt and perform consistently even under poor grazing conditions (Bisschoff and Lotriet, 2013). Due to their capacity to adapt and produce in a variety of production systems, indigenous South African breeds such as Afrikaner, Drakensberger, and Nguni have made significant contributions to livestock production (Abin *et al.*, 2016).

Significant genetic progress in growth and production traits have been made in most beef cattle breeds (Webb, 2006). Selection for growth has impacted the rate and the extent of underlying physiological processes that influence livestock growth and development (Webb and Casey, 2010) which led to unintended adverse results in livestock. One of the most dramatic effects of selection for growth in cattle was a mutation in the *MSTN* gene, especially the downregulation of *MSTN* expression which resulted in excessive muscling (Aiello *et al.*, 2018). The double muscling condition in beef cattle breeds such as the Belgian blue and Piedmontese is an extreme example of selection for growth in cattle (Sartelet *et al.*, 2012; Solé *et al.*, 2017).

The double muscling condition was first documented in the beginning of the nineteenth century by a British farmer named George Culley (Kambadur *et al.*, 1997; Fiems, 2012; Colombino and Giaccaria, 2015). In 1929, Wriedt suggested that the condition was due to a single gene defect, but the suggestion did not receive support until 1995 when the genetic defect was mapped to the centromeric end of bovine Chromosome (BTA) 2 by scientists in the Faculty of Veterinary Medicine at the University of Liege, Belgium (Charlier *et al.*, 1995). After their experiment, it was evident that the genetic defect was due to single, autosomal, gene defect (Flynn and Flynn, 2015).

Despite being discriminated against by most breed societies in South Africa, double-muscled cattle are accepted across the world due to certain advantages (Wiener *et al.*, 2009; Allais *et al.*, 2010; Webb and Casey, 2010). The double muscled animals are characterized by increased lean meat yield, low fat content, as well as significant improvement in carcass dressing percentage, resulting in high meat yield (Allais *et al.*, 2010; Fiems, 2012). Despite these advantages there are also some side effects associated with the condition. The adverse effects in animals that are double-muscled are variable, as breeds differ in terms of the causative alleles that they carry (Short *et al.*, 2002). Negative effects such as leg problems, macroglossia, weak bones, erythrocyte fragility, higher susceptibility to heat stress and lower reproduction capacity are often associated with muscular hypertrophy (Webb and Casey, 2010; Hu *et al.*, 2013; Miar *et al.*, 2014).

Currently there are nine known genetic variants that are included in routine DNA testing, namely Nt821 *(del11)*, Nt419 (C313Y), Nt414 (Nt748), E226X (E291X), Q204X, F94L, L64P, Nt267 (Nt324) and

S105C (D182N) that restrain and decrease the activity of myostatin protein that can cause double muscling. Some of these are breed specific (Glass and Spiegelman, 2012). These genetic variants have various levels of impact, some have more adverse effects than others. For example, nt821and Q204X are known as "detrimental genes" because they show more severe signs compared to F94L (Dunner *et al.*, 2003). It is therefore important to understand that different cattle breeds may carry different *MSTN* mutations, meaning that the resultant impact on muscular hypertrophy and meat quality vary for different genotypes (Webb and Casey, 2010).

Research on double muscling has most commonly been performed on the Belgian Blue, Piedmontese, and Limousin breeds, as they are known carriers of the muscular hypertrophy condition and show a high frequency of double muscling (Kambadur *et al.*, 1997). The double muscling phenotype in the Limousin breed is favourable while that of the Belgian Blue and Piedmontese is unfavourable. These breeds are characterized by significant improvements in marbling score, protein content and carcass dressing percentage (Fiems, 2012). Additionally, carcass from double-muscled animals have low fat content which contribute to the eating quality, flavour, and juiciness of the meat (Allais *et al.*, 2010). However, double-muscled animals are more prone to respiratory diseases, lameness, nutritional stress and heat stress which results in lower robustness (Aiello *et al.*, 2018). Coupled with this, reproductive performance-related problems are often associated with the muscular hypertrophy condition. Double-muscled cattle commonly undergo caesarean procedures during parturition to help with calf birth as they face calving difficulties (Kolkman *et al.*, 2010). If they are not assisted, chances of calf survival are reduced, and this can result in high mortality rates. The high degree of muscling in the pelvic area prevents pelvic distension, which further exacerbate the problem (Wiener *et al.*, 2002).

The general role of the *MSTN* gene in the expression of growth and muscle development is evident from literature, but the specific impact of various variants has not been quantified in SA beef breeds. South African farmers have indicated that double muscling was observed in their beef herds and subsequently requested a research project to identify the specific variants causing it, as well as its impact on economically important traits. Although the causative mutations for double muscling syndrome in cattle have been studied and documented in other countries, there is little or no evidence of its incidence in South Africa. This poses a challenge to farmers of not knowing whether to select for heterozygote carriers or to completely eradicate double muscling variants in their herds due to the conflicting reports available. Recognising the effect of *MSTN* on double muscling entails the need for a clear understanding of the relationship that exist between the *MSTN* gene and growth, carcass, and reproduction traits. The improved understanding of the role of *MSTN* in beef production is significant for SA beef industry for economic purposes. This research project endeavours to investigate the effect of *MSTN* on reproduction, growth, and carcass traits.

1.2 Aim and objectives

A number of South African beef breeds have reported the double muscling phenotype. Five breeds (Bonsmara, Beefmaster, Drakensberger, Brangus and Limousin) originally participated in a research project for the beef genomic program (BGP) funded by Technology Innovation Agency (TIA) where genotypes were generated for establishing reference populations for GEBV's. A pilot study from the BGP was extended with additional genotypes for Bonsmara and Drakensberger breeds. The Bonsmara and Drakensberger breeders continued with voluntary screening at the myostatin loci and has sufficient genotypes to evaluate the influence of myostatin variants on economically important traits. The primary aim of the study was to identify the causative variants for double muscling and quantify their effect on economically important traits in the SA Bonsmara and SA Drakensberger beef breeds.

The traits to be studied in this research include reproduction traits (age at first calving (AFC), inter-calving period (ICP), longevity, scrotal circumference (SC)); growth traits (direct birth weight (BW_{DIR}), direct weaning weight (WW_{DIR}), average daily gain (ADG) and feed conversion ratio (FCR)) and carcass traits based on real-time ultrasound (RTU) (fat, marbling, eye muscle area (EMA)).

The following objectives have been set:

1. To estimate the frequency of the most common variants of the *MSTN* mutation in five SA beef cattle breeds, using genomic data.
2. To perform an association study between the *MSTN* variants and reproduction traits in the Bonsmara and Drakensberger populations using GEBVs.
3. To perform an association study between the *MSTN* variants, growth, and carcass traits in the Bonsmara and Drakensberger populations using GEBVs.

Chapter 2 Literature review

2.1 Introduction

The availability of modern biotechnology (genomics) has made it possible to genotype farm animals to identify SNPs associated with genes and mutations that cause variations in traits of economic importance (Buermans and Den Dunnen, 2014). This technology has the ability to change selection strategies and increase genetic gains in farm animals (Cloete *et al.*, 2014). The advancement and commercial availability of various SNP arrays have supplied cattle farmers with an extra tool to use to increase selection accuracy and rate of genetic progress (Van der Westhuizen *et al.*, 2014). Myostatin (*MSTN)* gene causes double muscling syndrome also known as muscular hypertrophy. Muscular hypertrophy which leads to greater carcass value and growth rates has extended widely among numerous European cattle breeds (Bennett *et al.*, 2019). Due to changes in selection pressure, which varies depending on management requirements and market, the expression of muscular hypertrophy differ among different cattle breeds (Dunner *et al.*, 2003).

Prior to DNA tests development and the identification of the cause of muscular hypertrophy, cattle breeders based their selection on increased muscling (Arthur, 1995). Muscular hypertrophy is one of the genetic defects which can be tested for using commercial SNP arrays. Using genomics, a number of *MSTN* variants in several beef cattle breeds have been identified, which is a cause for concern in terms of animal welfare (van Marle-Köster and Visser, 2021). This section aims to review relevant literature regarding the underlying genetics of the *MSTN* gene, the physiological mechanisms involved in double muscling and the effect of this syndrome on the performance of various cattle breeds.

2.2 Organization of beef breeding in South Africa

In South Africa (SA), there are more than 30 cattle breeds used mostly for beef production. This includes British, European, composite and indigenous breeds (Van Marle-Köster *et al.*, 2013) farmed under various production systems and subjected to different breeding objectives (Lashmar *et al.*, 2019). The beef industry contributes 26.24% towards the livestock production industry (DAFF, 2021). Department of Agriculture, Forestry and Fisheries (DAFF) data indicated an increase in beef production over time. The number of cattle slaughtered per year in South Africa increased from 2.6 million in 1985/1986 to 3.2 million in 2019/2020. Additionally, the number of calves slaughter per year decreased from 144 000 in 1985/1986 to 18 000 in 2019/2020 in South Africa. In 1985/1986, per capita consumption in South Africa was 19.89 kg/year and it decreased to 18.12 kg/year in 2019/2020. The total amount of beef consumed in South Africa was 630 000t in 1985/1986 with an increase to 1.16 million tonnes in 2019/2022.

Within the commercial sector, the seedstock sector's sire-dam complementarity permits growth and carcass traits to dominate the breeding objectives and selection criteria. The terms “efficiency and

productivity" are the driving forces behind the beef industry and the performance of the enterprise is assessed based on economic returns (Van Marle-Köster *et al.*, 2013). Efficiency and productivity of a beef enterprise in the extensive grazing systems are influenced by good adaptation of animals to the prevailing environmental conditions (Mirkena *et al.*, 2010). The commercial sector primarily concentrates on increasing the number of weaned calves for a given number of cows, which will then be sold to the feedlot (Rust and Groeneveld, 2001).

SA is one of the few African countries with a nationwide program for animal recording for the genetic improvement of cattle breeds (van Marle-Kőster *et al.*, 2015). From the beginning of national animal recording programs in the 1960s, breeds such as the Bonsmara, Drakensberger, and Afrikaner participated in national animal recording, supplying pedigree and performance information for genetic evaluation (Marle-Köster *et al.*, 2021). In South Africa, the livestock industry has access to the Logix system, Breedplan and Agricultural Research Council (ARC) for animal recording services. Approximately 42% of cattle participating in the Logix Beef (SA Stud Book's animal recording database) is the SA Bonsmara, while 4.5%, 3.3%, 2.4%, and 5.6% are Drakensberger, Tuli, Afrikaner and Nguni, respectively (SA Stud Book, 2016). In livestock industry, breeds differ in terms of population size and recorded traits of economic importance due to specific breeding objectives for the breeds (Van Marle-Köster *et al.*, 2013). Table 2.1 summarizes average performance for reproduction and carcass (RTU based) traits of cattle types and breeds used in beef production in South Africa.

Table 2.1 A summary of early, intermediate, and late maturing beef cattle breeds in South Africa (SA Stud Book, 2016) along with their averages for the reproduction and carcass traits of interest in this study (SA Stud Book, 2021)

Breed	**Maturity Type**	**AFC (Months)**	**ICP (Days)**	**SC (mm)**	**Fat (mm)**	**Marbling (%)**	**EMA (mm^2)**
Bonsmara	Intermediate	31	405	347	5.50	2.54	72.0
Drakensberger	Intermediate	33	418	336	4.35	2.49	63.0
All Beef Type	Early, late	32	410	345	3.35	2.60	69.5
Sanga Type	Intermediate	33	407	330	4.40	2.63	62.0
Taurus Type	Early, late	33	413	348	5.40	2.85	66.5
Indicus Type	Late	34	450	332	5.70	2.50	63.0
Composite Type	Intermediate	31	403	346	5.45	2.55	71.5

Table 2.2 summarizes average performance for growth of cattle types and breeds used in the beef production in South Africa.

Table 2.2 A summary of early, intermediate, and late maturing beef cattle breeds in South Africa (SA Stud Book, 2016) along with their averages for the growth traits of interest in this study (SA Stud Book, 2021)

Breed	Maturity Type	BW (kg)	WW (kg)	MW (kg)	ADG (g)	FCR (kg/kg)
Bonsmara	Intermediate	35	220	514	1604	5.66
Drakensberger	Intermediate	34	196	487	1379.5	5.65
All Beef Type	Intermediate	35	218	508	1550	5.72
Sanga Type	Intermediate	32	187	455	1567.5	5.65
Taurus Type	Early, late	37	220	559	1626	5.44
Indicus Type	Late	29	185	421	902.5	6.44
Composite Type	Intermediate	35	223	515	1578.5	5.74

To set up breed reference populations that are appropriate for genetic evaluation, a larger number of genotyped animals is required. In the BGP, biological samples (such as hair samples) were collected from registered beef cattle that were participating in performance recording. The BGP as a large-scale project was established with the funding from Technology Innovation Agency (TIA, Department of Science and Technology). The overall aim of the BGP was to introduce genomic improvement in the beef population in South Africa (Becker, 2016). The TIA funded initiative allowed accumulation of approximately 7 000 genotypes across 16 beef cattle breeds for the period of three years (2015-2018) of routine genotyping (van Marle-Köster and Visser, 2018).

For diagnostic testing, verified SNPs are included in commercial SNP panels. There are several genetic defects affecting livestock that are identified using diagnostic tests and some of them were added on commercial SNP arrays as some were single gene-based tests (van Marle-Köster and Visser, 2021). Double muscling is also among genetic defects included on these SNP arrays. Through diagnostic tests, genetic defects that have a significant effect on the economy can be identified to eradicate the causative variant and be removed from breeding population completely (Venhoranta *et al.*, 2014). Table 2.3 summaries a number of genetic defects that are available on SNP arrays, which can be requested during routine genotyping in cattle.

Table 2.3 Summary of available SNP arrays available for *MSTN* variants genotyping in cattle (Doyle *et al.*, 2020)

SNPs Array	Number of SNPs
Bovine Illumina SNP50	54 001
Illumina high density	777 962
Illumina low density	6909
Illumina 3k panel	2900
Bespoke genotype panel (IDB)	
Version 1	17 137
Version 2	18 004
Version 3	53 450

Before the advancement of the genomic technologies, diagnostics of animals that exhibited extreme double muscling phenotype were based on phenotype assessment, which was accurate for extreme phenotypes but not for heterozygous carrier detection (O'Rourke, 2010). This is due to the fact that heterozygous carriers for the double muscling syndrome closely resemble the homozygous normal animals (Charlier *et al.*, 1995) especially in terms of female reproduction (Casas *et al.*, 1999; Casas *et al.*, 2004). Therefore, having accurate diagnostic tests to identify heterozygotes of this phenotype was an ideal solution to avoid breeding challenges that double muscling syndrome possess for farmers. In addition, to eliminate genetic disorders such as double muscling syndrome from the respective animal populations by identifying heterozygotes and preventing them from mating with animals that are heterozygotes for the same genetic defect, diagnostic testing is utilized (van Marle-Köster and Visser, 2021). In South Africa, there are a number of laboratories for diagnostic testing that are relatively cost effective for use in both the commercial and emerging farmer sectors (van Marle-Köster and Visser, 2018). Table 2.4 summarises the laboratories and the diagnostic tests that are available in South Africa.

Table 2.4 Diagnostic tests available for ruminants in South African laboratories (van Marle-Köster and Visser, 2018)

Diagnostic tests	Type of species	South Africa laboratories
DNA profile	Cattle, sheep, goats	Unistel, Onderstepoort veterinary genetics lab, Clinomics, GENEdiagnostics
Parentage	Cattle, sheep, goats	Unistel, Onderstepoort veterinary genetics lab, Clinomics, GENEdiagnostics
3-in-1 DNA/Pompes/CMS	Cattle	Unistel, Onderstepoort veterinary genetics lab, Clinomics
Cytogenetics: 1/29 Translocation	Cattle	Unistel
Double muscling/Myostatin	Cattle	Unistel, Clinomics
Curly calf syndrome	Cattle	Unistel
Polled, scurred, horned	Cattle	Unistel
Bulldog mutation screening	Cattle	Unistel, Clinomics
Freemartin	Cattle	Unistel

These tests are mostly based on microsatellite technology.

SA breeders have access to BLUP-EBVs and genomic holds potential for GEBVs. Bonsmara, Drakensberger, Beefmaster and Hereford received GEBV's (SA Studbook, 2017). Routine genotyping offers potential for diagnostic testing for genes including polledness and *MSTN*.

2.3 Genetic basis of the double-muscled phenotype

Increased muscle mass in domestic animals is one of the key breeding goals, as it correlates to higher carcass yield in livestock. Before the availability of molecular technology, the genetic basis of the phenomena of "double-muscled" animals was completely unknown (Matsakas and Diel, 2005). Through the advancement of transgenic technology, scientists were able to produce "knockout" mouse models that allow them to effectively investigate the biochemical pathways and mechanisms of gene action (Matsakas and Diel, 2005). The genetic basis of double muscling syndrome created a controversy between researchers. Before it was challenged by multiple theories, the syndrome was reported to be caused by a single gene determinant (Wriedt, 1929). The evidence from animal breeding researches strongly indicated that double muscling was monogenic and autosomal (Charlier *et al.*, 1995), however, the observation of this condition in numerous cattle breeds fuelled doubts regarding the homogeneity of the double muscling locus. Researchers were uncertain about the precise mode of inheritance of the double muscling syndrome until Hanset and Michaux (1985) classified it as partially recessive. Other researchers (Dunner *et al.*, 1997; Grobet *et al.*, 1997; Grobet *et al.*, 1998; Berg and Shahin, 2019; Matika *et al.*, 2019) also reported that

double muscling syndrome has a recessive mode of inheritance. This was supported by the observation of the heterozygous carrier animals since they have growth traits similar to those of homozygous normal and homozygous affected animals (Arnold *et al.*, 2001).

In 1995, the muscle hypertrophy (*mh*) locus was localized to bovine chromosome (BTA) 2 by linkage analysis, providing strong evidence that the *mh* locus described the single, autosomal, major gene underpinning the double muscling phenotype (Charlier *et al.*, 1995). Among the European breeds, muscle hypertrophy became increasingly widespread and was termed "double muscling" (Konovalova *et al.*, 2021). This happened after the muscle hypertrophy was described in depth, and it changed the farmer's perceptions of carcass traits, marking the beginning of new era of animal breeding with increasing muscle mass (Konovalova *et al.*, 2021). An F1 backcross of supposed hemizygous cattle to known homozygous knockouts, which produced a 1:1 ratio of double-muscled to normal offspring, was used to confirm simple mono-factorial Mendellian segregation (Arnold *et al.*, 2001).

The localization of the *mh* locus on BTA 2 was performed in the Belgian Blue breed. During the first evaluation, Charlier *et al.* (1995) constructed a marker map of BTA 2 and examined the relative rates of recombination between markers after finding no initial evidence to support the association of the *mh* locus to any specific autosome. As a result, the marker with the shortest physical distance from the *mh* locus and the lowest incidence of recombination was located on BTA2 at the centromeric end of the BTA2 linkage group. Since the *mh* locus has been mapped, marker-assisted selection can be used to select for or against double muscling phenotype depending on the breeding goals of the various populations (Charlier *et al.*, 1995). Research indicated two alleles that were involved in the expression of muscular hypertrophy, namely the wild type (+) and mutated (*mh*) *MSTN* alleles (Coopman *et al.*, 2007; Allais *et al.*, 2010; Widyas *et al.*, 2018; Lee *et al.*, 2019). Three genotypes segregated at this particular locus in the populations of Belgian Blue and Piedmontese, namely conventional cattle/wild type (+/+), heterozygous (*mh*/+) and homozygous (*mh*/*mh*) (Pozzi *et al.*, 2009; Allais *et al.*, 2010; Lee *et al.*, 2019). Figure 2.1 demonstrate the effect of mating a heterozygous carrier bull and heterozygous carrier dam.

Figure 2.1 Illustration of the of the effect of mating heterozygous carrier bull with heterozygous carrier dam

The discovery of the *MSTN* gene and protein, as well as the conclusive association between mutant MSTN and the double-muscled phenotype, had an impact that went beyond simply identifying the mechanism of muscle cell hyperplasia. It is now possible to directly genotype individuals, distinguishing between heterozygous carrier and double-muscled animals, as opposed to the previous method of physical observation (Konovalova et al., 2021; Vinet et al., 2021; Ceccobelli et al., 2022). Being able to recognize these variations between species and breeds enables a better understating on how minor alterations in a protein's molecular structure can have a significant impact on its functionality (Arnold et al., 2001).

2.4 Characterization of the myostatin (*MSTN*) gene structure

Myostatin which is also known as the growth and differentiation factor 8 (*GDF-8*), is a protein that is released and produced by the myocytes to inhibit muscle growth (myogenesis) in young animals (Lee, 2004; Elkina *et al.*, 2011; Sharma *et al.*, 2015). This protein is also involved in regulating muscle mass homeostasis (Dominique and Gérard, 2006) and regulation of adipogenesis (Deng *et al.*, 2017). During the last decades, *MSTN* with different genetic variants were confirmed as the cause for the double muscling syndrome (Ménissier, 1982; Arthur, 1995). The genetic variants inactivate the gene leading to the loss of ability to stop the growth of muscle fibre in livestock (McPherron and Lee, 1997; Grobet *et al.*, 1998) and decrease functional *MSTN* production. Double muscling syndrome is present in several mammalian species such as mice, dogs, cattle, chickens, pigs, horse and in humans (Han *et al.*, 2013; Aiello *et al.*, 2018). The MSTN protein's molecular structure is conserved among vertebrates, with C-terminal sequences that are 100% similar in mice, rats, humans, pigs, and chickens as well as in cattle (McPherron and Lee, 1997). This has sparked interest in developing a gene-marker test to determine an animal's genotype (Rasmussen, 2016). In several species such as sheep and pigs, the impact of *MSTN* variability on productivity traits in farm animals have been studied (Konovalova *et al.*, 2021). Polymorphisms in the promoter region of the *GDF-8* gene, in particular, affect meat colour and ultimate pH, affecting meat quality, growth and reproduction traits in several cattle breeds (Fiems, 2012; Druet *et al.*, 2014; Flynn and Flynn, 2015).

Myostatin, a potent inhibitor of muscle development, is an evolutionary conserved member of the transforming growth factor β (TGF-β) superfamily (Breitbart *et al.*, 2011; Elashry *et al.*, 2012; Hennebry, 2014). This superfamily of signalling proteins (cytokines) plays an important role in regulation of embryonic development and maintenance of tissue homeostasis in adult animals (Hickford *et al.*, 2010). In addition, the sequence and the structural pattern that define the members of the TGF-β superfamily of signalling cytokines determine their function (PIEK *et al.*, 1999). Myostatin is significantly different from other member of this family more especially its C-terminal region (Arnold *et al.*, 2001).

The researchers in the Department of Molecular Biology and Genetics at the Johns Hopkins University School of Medicine in Baltimore used molecular genetic techniques to investigate genes that are

responsible for TGF-β (Rasmussen, 2016). The TGF-β superfamily sequences are highly conserved across species and encode a secretion signal sequence, a proteolytic processing site and a conserved sequence of cysteine residues in the C-terminal end (McPherron and Lee, 1997). The peptides in this family are characterized by conserved sequences of nine cysteine residues at the C-terminal end as shown in Figure 2.2. This led the Johns Hopkins researchers to the discovery of the unidentified gene which was initially called *GDF-8*. This gene coded for a novel protein containing 376 amino acids which was produced in developing adult skeletal muscles specifically (Flynn and Flynn, 2015). The *GDF-8* was later renamed as *MSTN* as it functions specifically as a negative regulator of skeletal muscle growth (McPherron and Lee, 1997). *MSTN* as an active member of TGF- β family is a 26 kilodalton (kDa) homodimeric protein that is expressed during embryogenesis in myotome layer of the somites that is developing and expressed later in all skeletal muscles (Matsakas and Diel, 2005).

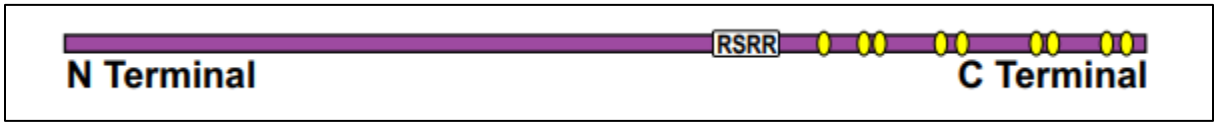

Figure 2.2 Myostatin gene: conserved regions (cysteine positions are indicated in yellow) (Arnold *et al.*, 2001)

Due to the phenotypic similarity between mice whose *MSTN* gene had been knocked out and double-muscled cattle, the John Hopkins scientists analysed the *MSTN* gene of the double-muscled animals, which was defective with a deletion of eleven (11) base-pairs (Grobet *et al.*, 1997; McPherron and Lee, 1997) to find the biological function and the location of *GDF-8* (Rasmussen, 2016). This resulted in the production of non-functional *MSTN* which caused the double muscling syndrome. The *MSTN* gene was discovered to have different variants that cause loss of the function of this gene. The functional loss observed in the Piedmontese breed is caused by one of the nullifying mutations, C313Y, which converts the fifth of the nine cysteine residues in mature *MSTN* to tyrosine, see Figure 2.2.

The *MSTN* gene is made up of three subunits, namely the C-terminal peptide, signal sequence, and N-terminal peptide (Dominique and Gérard, 2006; Rasmussen, 2016), see Figure 2.3. This gene consists of three exons and two introns, and is located at 2q11-q12 position on BTA 2 (Tellam *et al.*, 2012). Additionally, the *MSTN* gene has been reported to influence muscle metabolism and gene expression (Hocquette *et al.*, 2007). The length and sequence of the *MSTN* gene differ slightly across species (Rasmussen, 2016). Variation in the bovine *MSTN* gene have been documented in different cattle breeds. This includes variation in the *MSTN* coding and promoter regions of the double-muscled cattle. As a result, in the coding region, six genetic variants (Nt419, Nt821, Q204X, E226X, E291X and C313Y) were identified in exon 2 and exon 3 of *MSTN* in various cattle (Rasmussen, 2016). In addition, variation was also described in the promoter region, upstream of the *MSTN* coding sequence (Hill *et al.*, 2010).

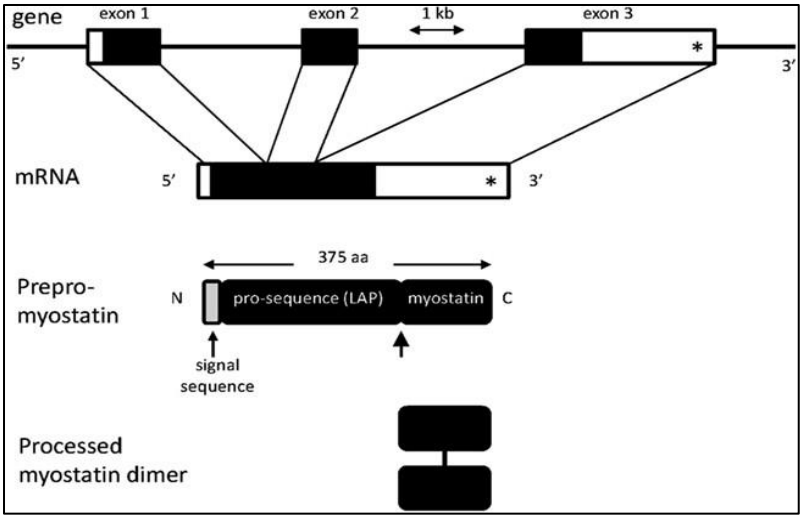

Figure 2.3 Schematic representations of *MSTN* gene and protein structures, (Tellam *et al.*, 2012). Unshaded regions in the exons and mRNA represent the 5' and 3' untranslated regions. Lightly shaded region in the prepro-myostatin protein sequence corresponds to the signal sequence. Thick arrow shows site for processing of the promyostatin protein. The mature *MSTN* polypeptide forms a dimer held together by a disulphide bond.

MSTN including its propeptide was discovered to exists as a large, latent complex with other proteins (Arnold *et al.*, 2001; Lee and McPherron, 2001; Matsakas and Diel, 2005). *MSTN* binding proteins inhibit the activation of the *MSTN* or serve as *MSTN* receptor binding (Dominique and Gérard, 2006). *MSTN* receptors, as with the other TGF-β superfamily are divided into two sub-families namely type I and type II receptors (Massague, 1998). Table 2.5 summarises the elements of the TGF-β ligand family pathway. The active *MSTN* peptide usually bind to a type II receptor kinase first as this activates a type I receptor kinase then Smad proteins will be phosphorylated (Novianti, 2011).

Table 2.5 Summary of the elements of the TGF-β ligand family pathway (Dominique and Gérard, 2006)

Ligand	Type II receptor	Type I receptor	R-Smad
Activins	ActRII	ActRIB/ALK-4	Smad 2
Myostatin	ActRIIB	Unknown	Smad 3
TGF-β	TβRII	TβRI/ALK5, ALK1, ActRI/A1K3	Smad 1
BMPs	BMPRII	BMPRI/ALK3	Smad 5
GDFs	ActRII, ActRIIB	BMPRIB/ALK6, ActRI/ALK2	Smad 8

The specific role of the Smad proteins is to regulate the expression on many other downstream proteins (Lee, 2004). There are several other proteins that have been reported to interact with the *MSTN* and it is likely that there are other proteins that have not been identified (Novianti, 2011). These proteins

are affected during the muscle mass regulation by the *MSTN* (Dominique and Gérard, 2006). The *MSTN* does not only act as a major gene that controls muscle growth but also interact with other genes (Widyas *et al.*, 2018).

2.5 Myostatin signalling pathways and its control of skeletal muscle development

Given the overview of the *MSTN* genetic basis, structural characteristics, and physiological function, *MSTN* was first considered as a limiting factor in normal muscle development (Arnold *et al.*, 2001) and as growth regulator in early development (McPherron and Lee, 1997). Myostatin is expressed most predominantly in skeletal muscle, but it can also be expressed in mammary glands, and other tissues such as cardiomyocytes (Ji *et al.*, 1998). Additionally, it is expressed to a lesser extent in adult muscle tissue, but highly expressed in embryonic and foetal stages (Arnold *et al.*, 2001). Xu *et al.* (2003) reported that the expression of the myostatin in skeletal muscle appear to occur in certain type of the muscles. They indicated that expression of myostatin mRNA was primarily expressed in red muscles, while the expression in white muscles was to a lesser extent. Glucocorticoid-induced muscle was associated with upregulation of myostatin expression while regenerating muscle was linked to down-regulation of myostatin expression (Arnold *et al.*, 2001).

Myostatin as the member of the TGF-β family is an extracellular cytokine, it mediates the signal through activin receptors (Morikawa *et al.*, 2016; Nickel *et al.*, 2018). Myostatin is produced and secreted by muscle cells, and it activates multiple pathways by signalling through the activin-receptor IIB/ALK 4/5 heterodimer, leading to a reduction in muscle growth and muscle differentiation (Elkina *et al.*, 2011; Fakhfakh *et al.*, 2011; Rodriguez *et al.*, 2014), see Figure 2.4. Signalling of the *MTSN* through ActRIIB is pivotal for the regulation of muscle growth. However, the binding of the *MSTN* to the ActRIIB in serum can be prevented by Follistatin protein, an extracellular cysteine-rich glycoprotein with a structure distinct from TGF-family members (Elkina *et al.*, 2011). Another protein that can inhibit the binding of the *MSTN* is the growth and differentiation factor-associated serum protein 1 (GASP-1) (Casas *et al.*, 2000), unlike other members of the Follistatin family, GASP-1 has no affinity for activin (Hill *et al.*, 2003).

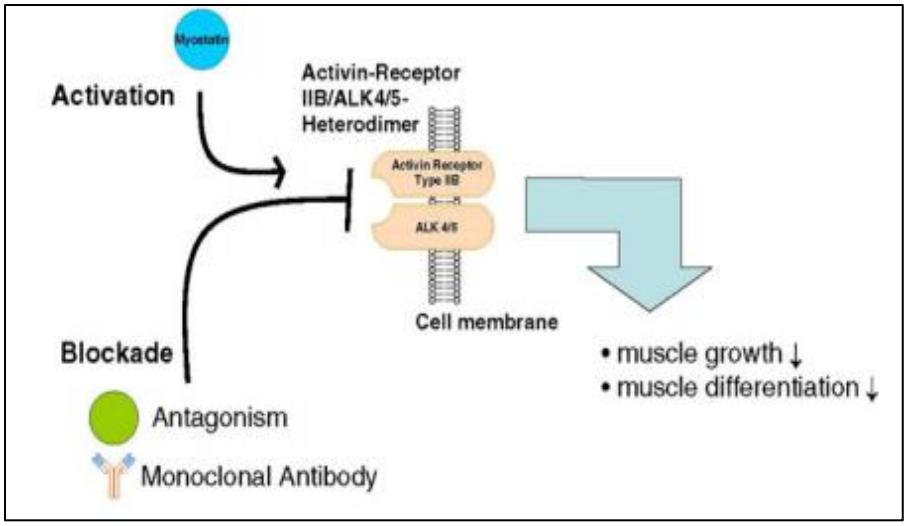

Figure 2.4 The schematic mechanism of myostatin extracellular signalling (Elkina *et al.*, 2011)

Fakhfakh *et al.* (2011) proposed the model illustrated in Figure 2.5 to show the effect of the presence of dominant negative mutant of ActRIIB (dnActRIIB) where *MSTN* binds to the dnActRIIB receptor without triggering signal transduction in myoblasts. Figure 2.5a shows that mature *MSTN* binds to the dimer activin receptor type IIB (ActRIIB) (1) then, the ActRIIB recruits the activin receptor type I (ActRI) (2), followed by the activation of the kinase activity of ActRI by transphosphorylation (3), then Smad2 and Smad3 are subsequently activated through phosphorylation (4), thereafter; Smad4 forms a complex with the Smad2–Smad3 heterodimer (5), then the complex translocate into the nucleus (6), Interaction with different cellular partners in order to regulate the transcription of various downstream response genes (7 and 8). Figure 2.5b illustrate the binding of the mature *MSTN* to the dimer dnActRIIB (9) then due to lack of the kinase domain of dnActRIIB, ActRIIB will not recruit or activate the ActRI (10). Thus, there is no activation of Smad protein function and a downregulation of specific gene expressions (11).

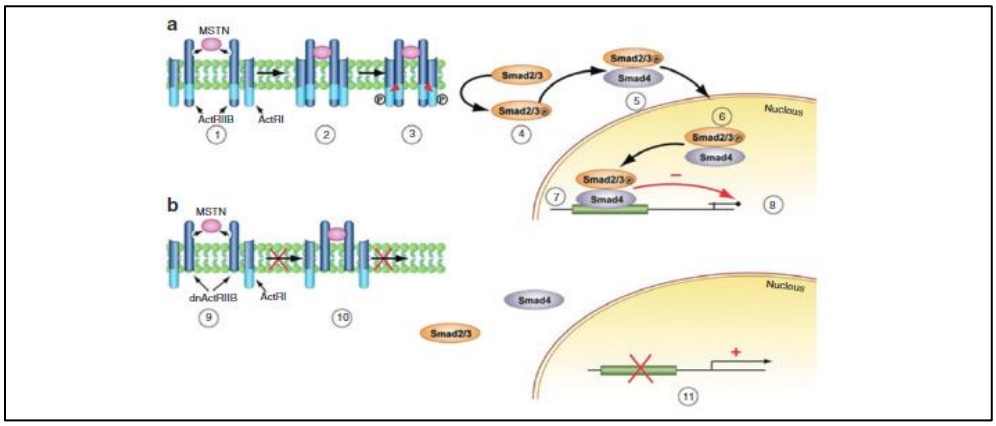

Figure 2.5 Blocking the myostatin signal with a dominant negative receptor (Fakhfakh *et al.*, 2011)

MSTN also blocks the growth of the myoblast within the cell by inhibiting the expression of myogenic differentiation (MyoD), the myogenic regulatory factors (MRF) and by stimulating the expression of p21, the cyclin-dependent kinase inhibitors (Rodriguez *et al.*, 2014). MyoD is a highly arranged sequential program that generates skeletal muscle and regulates the expression of the *MSTN* during myogenesis (Aiello *et al.*, 2018). In addition, MyoD is a highly proliferative precursor of the muscle (as indicated in Figure 2.6) that emerge during embryogenesis differentiation into myoblasts. MRFs regulate the activation of muscle differentiation-specific genes and controls the determination and differentiation of skeletal muscle cells during postnatal myogenesis and embryogenesis (Hernández-Hernández *et al.*, 2017). These MRFs (Myf5, MyoD, MRF4 and Myogenin) are members of basic helix-loop-helix transcription factors (Asfour *et al.*, 2018; Kazim *et al.*, 2019). Additionally, the MRFs form a group of muscle proteins that act at several points in the lineage of muscle to establish the phenotype of the skeletal muscle through regulation of proliferation (Hernández-Hernández *et al.*, 2017).

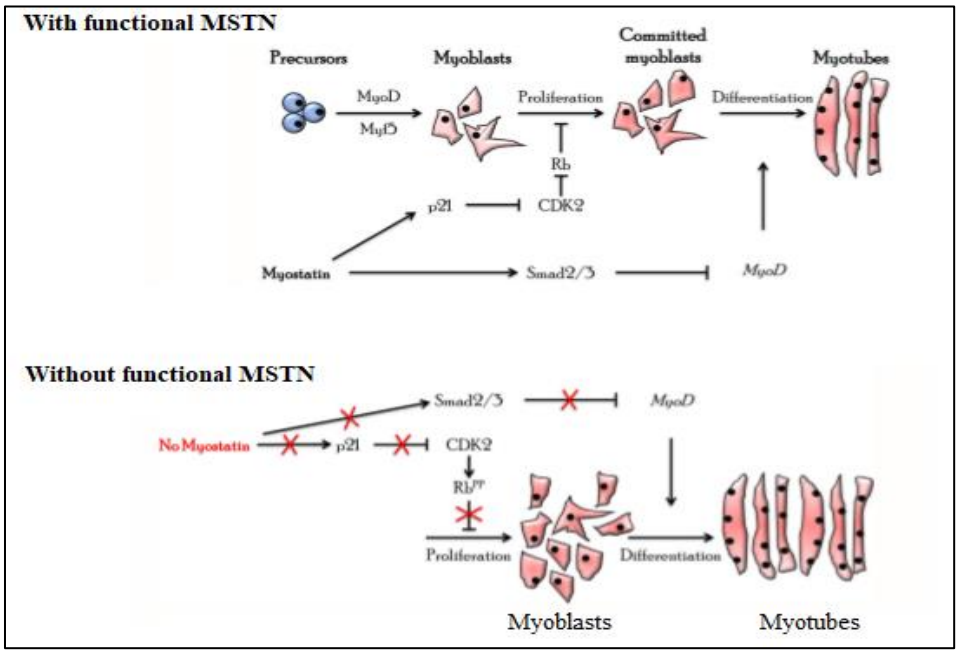

Figure 2.6 Myostatin action during myoblast proliferation and differentiation (Aiello *et al.*, 2018)

Functionally, mature *MSTN* regulates muscle development during muscle precursor proliferation, myoblast proliferation and differentiation (Aiello *et al.*, 2018). During the proliferation of MyoD-expressing myoblasts, p21 which is cyclin-dependent kinase inhibitor is upregulated by myostatin signalling (Thomas *et al.*, 2000; Spiller *et al.*, 2002). The role that the p21 plays is to regulate and inhibit the activity of cyclin-dependent kinase-2 (cdk2) (Rodriguez *et al.*, 2014; Rasmussen, 2016). Subsequently, cdk2 aim for retinoblastoma protein (Rb) which affects E2F transcription factors that are important for progression of the myoblasts from the G_1 to S-phase in the cell cycle (Thomas *et al.*, 2000). Furthermore, Arnold *et al.* (2001)

stated that the progression of the myoblasts through the cell cycle and cell cycle arrest are often controlled by cyclin-dependent kinase (CDK) and cyclin-dependent kinase inhibitor (CDI) complexes. *MSTN* as a negative regulator works through Rb-independent pathways and can inhibit the expression of MyoD, which is a requirement for myoblast terminal differentiation (Asfour *et al.*, 2018). The absence of MyoD slows down the proliferation to differentiation transition time (Yablonka-Reuveni *et al.*, 1999). This can explain the double muscling syndrome caused by the genetic variants in the *MSTN* gene that results in muscular hypertrophy and muscle hyperplasia as the restrictions that inhibit myoblast proliferation are removed.

2.6 Genetic variants of myostatin gene in beef cattle breeds

Up to 20 mutations have been identified in the bovine *MSTN* gene. Although most of these mutations doesn't show any effect due to some parts of the gene being inactive; these mutations are economically important. Myostatin activity is completely or partially lost as a result of nucleotide changes, which leads double-muscled phenotype (Grobet *et al.*, 1997; Ceccobelli *et al.*, 2022). Nine genetic variants that are responsible for causing double muscling in cattle have been documented in literature (Bellinge *et al.*, 2005). Their impact on the double muscling phenotype, as well as on other traits, differ significantly. Some breeds carriers one mutation whereas other breeds carry more than one mutation.

These genetic variants include deletions, insertions, or nucleotide substitutions that limit the production or the activity of the *MSTN* gene. Several genetic variants in all *MSTN* coding regions (exons 1-3) have been characterized as silent and causing non-synonymous changes (Hunt *et al.*, 2009; Konovalova *et al.*, 2021). All the mutations that are found in the three exons and two introns of the *MSTN* gene are indicated in Figure 2.7, with green being silent mutations, blue being missense but not disruptive and red being disruptive mutations.

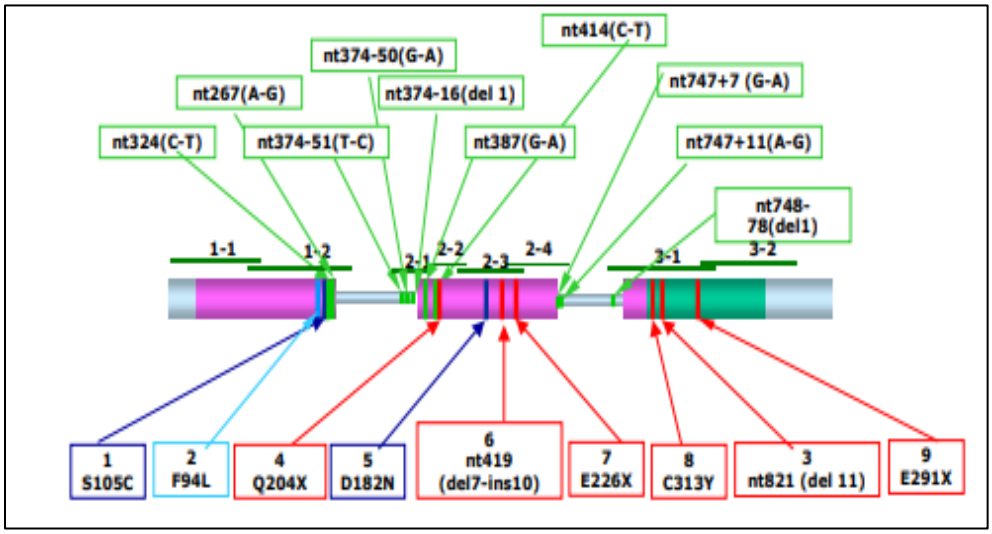

Figure 2.7 Schematic representation showing the exons and introns of the *MSTN* gene and approximate location of the most important mutations (Dunner et al., 2003)

The animal's appearance changes as a result of the changed gene's product. For instance, the double muscling syndrome result from the *MSTN* gene's product being non-functional or only partially functioning. Different SNPs that are directly related to the double muscling phenotype were identified in the *MSTN* gene of different cattle breeds. A summary of the common detected genetic variants that have been reported to be involved in the production of disrupted *MSTN*, resulting in double-muscled cattle, are indicated in Table 2.6.

Table 2.6 Summary of the major genetic variants in the myostatin gene of different cattle breeds (Konovalova *et al.*, 2021)

SNP*	Genetic variant	Amino Acid Change	Breed
Nt821(del11)	c.821–831del11	p.Glu275ArgfsX14	Asturiana, Belgian Blue, Blonde d' Aquitaine, Limousin, Parthenaise, South Devon, Santa Gertrudis, Braford, Murray Grey, Angus
Nt267	A > G	Silent mutation	Aubrac, Bazadaise, Salers
Nt324	C > T	Silent mutation	Asturiana
Nt387	G > A	Silent mutation	Asturiana, Salers, Galloway
C313Y	c.938G > A	p.Cyc313Tyr	Gasconne, Piedmontese, Parthenaise
E226X	c.610G > T	p.Glu226X	Maine-Anjou, Marchigiana
E291X	c.871G > T	p.Glu291X	Maine-Anjou, Marchigiana
F94L	c.282C > A	p.Phe94Leu	Angus, Limousin
Q204X	c.610C > T	p.Gln204X	Blonde d'Aquitaine, Charolaise, Limousin
S105C	c.314C > G	p.Ser105Cys	Parthenaise
L64P	c.191T > C	p.Leu64Pro	German Gelbvieh, Glanrind, Limpurger
D182N	c.544G > A	p.D182N	Asturiana

*Single nucleotide polymorphism

Most extensive studies to investigate double-muscling phenotype and its effects have been performed on the Belgian Blue cattle breed. Belgian Blue cattle originated from Belgium and were developed into dual purpose animals in the beginning of the 20th century (Druet *et al.*, 2014; Agung *et al.*, 2016). This breed was systematically selected for muscular hypertrophy to a point where the phenotype was virtually fixed in many herds (Charlier *et al.*, 1995; Kambadur *et al.*, 1997). Since the fixation of the phenotype in the 1980s, Belgian Blue cattle became popular for their exceptional muscular development or "double muscling" (Fasquelle *et al.*, 2009; Druet *et al.*, 2014). This phenomenon contributed to the global popularity of the Belgian Blue in crossbreeding programs (Agung *et al.*, 2016). The most common *MSTN* genetic variant in the Belgian Blue is the nt821 variant. Even though nt821 mutation is found in other breeds

too, the Belgian Blue breed was reported to be the only breed to be genetically homogeneous (Bellinge *et al.*, 2005). This has been reported by evaluating the diversity of haplotypes in different animal species (Dunner *et al.*, 2003).

Nt821 variant is one of the two disruptive mutations found in the 3rd exon on the *MSTN* gene which truncates the bioactive part of the protein (Grobet *et al.*, 1997; Kambadur *et al.*, 1997; Grobet *et al.*, 1998). The other mutation is the C313Y that changes cysteine to tyrosine in a highly preserved protein cysteine-knot structural motif region (Aiello *et al.*, 2018) resulting in complete loss of function (Grobet *et al.*, 1997; Kambadur *et al.*, 1997; Vinet *et al.*, 2021). The Piedmontese breed has been routinely selected for the double muscling condition to a point of fixation, similar to the Belgian Blue breed (>98 percent homozygosity in the Piedmonte region of Italy) (Miretti *et al.*, 2011; Aiello *et al.*, 2018). According to published research, the Piedmontese cattle breed carries not only the C313Y mutation but also the F94L mutation (Grisolia *et al.*, 2009; Bongiorni *et al.*, 2016).

The exon 3 region of the *MSTN* gene of cattle breeds also mutated, resulting in the *mh*- dominant allele, which increases meat yield by 20% and reduces carcass fat content by 50% (Marchitelli, 2003). In such cases, the mutated allele is strongly expressed, resulting in an irregular transcript consisting of a 41-bp inclusion between exons 2 and 3 with a premature termination codon that is expected to convert into a protein lacking the entire bioactive region (Aiello *et al.*, 2018). Mutations that were found to be disruptive resulting in premature stop codon includes, E291X in the 3rd exon of the homozygous affected Marchigiana animals (Grobet *et al.*, 1998; Dunner *et al.*, 2003) and Q204X, Nt419, and E226X variants in the 2nd exon of homozygous affected Charolais or Maine-Anjou animals (McPherron and Lee, 1997; Smith *et al.*, 2000; O'Rourke, 2010; Bongiorni *et al.*, 2016).

Within the *MSTN* gene of Limousin and Blonde d'Aquitaine breeds, Grobet *et al.* (1998) determined the loss-of-function mutation called F94L categorised as missense mutation. The polymorphism of *MSTN* gene of these two breeds is caused by the F94L variant, a nucleotide transversion of C to A at position 282 of exon 1 which determines the conservative phenylalanine-to-leucine substitution in the N-terminal latency-associated peptide (Grisolia *et al.*, 2009; Lines *et al.*, 2009; Allais *et al.*, 2010). The F94L variant was found to occur at a high rate (90-100%) in the Limousin breed, and was very scarce in Simmental (0.8%), Piedmontese (2%), Droughtmaster (4%), Rubia Galega x Nellore (2.3%) and Canchim (2.3%) breeds or absent in other breeds (Dunner *et al.*, 2003; Curi *et al.*, 2012; Lee *et al.*, 2015; Anwar *et al.*, 2020). This shows that there are factors yet unidentified causing muscular hypertrophy, perhaps situated on the outside of the *MSTN* coding region (Bellinge *et al.*, 2005). Animals that have two copies of F94L (homozygous affected) exhibit the increased overall yield of retail beef by up to 8% as well as the primal cut weight by up to 19% (Hayes, 2016). This results in better feed conversion rates. Some *MSTN* variants had similar action; for instance, F94L variant had similar effect as that of other variants such as Nt821

variant traits on traits such as fat. However, F94L variant does not have any effect on calving ease and birth weight, and breeders can use F94L *MSTN* polymorphism as possible genetic marker (Konovalova *et al.*, 2021). In research, there is a strong evidence that shows that F94L variant provides intermediate and useful phenotype compared to severe double muscling phenotype, which is expected to be a significant value in beef cattle industry (Sellick *et al.*, 2007; Esmailizadeh *et al.*, 2008).

In addition to disruptive mutations in coding regions is the recently discovered mutation in the 2nd intron of the *MSTN* gene called T3811>G3811 (Vinet *et al.*, 2021). The T3811>G3811 mutation was found to be the cause of an aberrant transcript and could be considered as a disruptive mutation just like other disruptive mutations in the exons of the *MSTN* gene (Bouyer *et al.*, 2014). The T3811 > G3811 mutation was only discovered in the Blonde d'Aquitaine breed in a study including 445 animals of 24 other breeds and it was almost fixed in the breed (Vinet *et al.*, 2021). The expression of the normal transcript in homozygous affected animals was observed and there was speculation that the T3811>G3811 variant may not be completely a disruptive mutation and may prevent excessive muscle hypertrophy (Bouyer *et al.*, 2014). Animals carrying the T3811>G3811 variant in their *MSTN* gene exhibit outstanding muscularity but not muscular hypertrophy similar to that observed in extreme double-muscled cattle in various other breeds (Bouyer *et al.*, 2014). In addition, T3811>G3811 variant has a similar effect to other *MSTN* genetic variants despite the fact that homozygous affected Blonde d'Aquitaine animals do not display extreme double muscling syndrome (Vinet *et al.*, 2021).

The *MSTN* gene has been found to be highly polymorphic in cattle, with the different polymorphisms being breed specific (Hu *et al.*, 2013). This is similar to other animal species (Aiello *et al.*, 2018). The heterozygous carrier animal tends to be largely average, while the homozygous recessive animals exhibit the double muscling phenotype (Wiener *et al.*, 2009). Different studies estimated the effect of various disruptive genetic variants on muscle hypertrophy and traits of economic importance in commercial populations or designed cross experiments. This includes the effect of Nt821 (Casas *et al.*, 2004; Gill *et al.*, 2009; Wiener *et al.*, 2009), Q204X (Allais *et al.*, 2010), E291X (Marchitelli, 2003; Sarti *et al.*, 2014), Nt419 (Kambadur *et al.*, 1997; McPherron and Lee, 1997; Grobet *et al.*, 1998), E226X (O'Rourke, 2010) and C313Y (Short *et al.*, 2002). In addition, the effect of the F94L which is a nondisruptive was also estimated (Esmailizadeh *et al.*, 2008; Cushman *et al.*, 2015; Bennett *et al.*, 2019). Table 2.7 shows the *MSTN* variants and their effects on homozygous affected and heterozygous carrier individuals. There is some consistent evidence that heterozygosity for double muscling syndrome produces an “intermediate” phenotype (Arthur *et al.*, 1989; Casas *et al.*, 1999; Smith *et al.*, 2000; O’Rourke *et al.*, 2009) with good carcass traits compared to homozygous but not superior to homozygous affected for double muscling syndrome (O'Rourke, 2010). Based on the myostatin haplotype, recessive individuals reflect incomplete dominance, incomplete penetrance, or modifier genes.

Table 2.7 Description of the effect of genetic variants in homozygous affected and heterozygous carrier animals (SA Stud Book,2019)

Mutations	Homozygous affected animals	Heterozygous carrier animals
Nt821 'Nt Gene'	Extremely heavily muscled, abnormally large, wide, and rounded rump and thighs, prominent creases between muscle groups. Little fat covering, thin bones and increased birth weights.	Recessive gene: carrier animals show quality carcass characteristics such as high rates of tenderness and reduced fat. Animals are less likely to be affected by more calving difficulties.
Q204X 'Q Gene'	Larger loin depth, reduced fat cover, and greater meat tenderness. Increased birth weights in increase the risk of difficult calving. Females have slightly reduced milking ability.	Partially dominant: carrier animals exhibit quality carcass traits but are less likely to be affected by reduced milking ability and larger birth weights.
F94L 'F Gene'	Larger loin depth, reduced fat cover, and greater meat tenderness. Heavier birth weights increase the risk of difficult calving. Females have slightly reduced milking ability.	Partially dominant: carrier animals exhibit the same characteristics as the homozygous animals but not to the same extent
E226X, E291X, C313Y, Nt419	Rare, causes double muscling, heavy birth weights, increased calving problems, and tender meat.	Recessive
S105C, D182N	Rare, causes increase in muscularity and reduce fat and marbling, with no change in birth weight.	Recessive

mh mutations occur sporadically, especially as selection pressure increases. Positive selection pressure for nonsynonymous mutations within the myostatin gene family existed around the time of the divergence of cattle, sheep, and goats, according to phylogenetic research, and these positive selective pressures on non-ancestral *MSTN* were relatively recent (Konovalova *et al.*, 2021). Managing double-

muscled cattle poses challenges for breeders such as calving difficulties due to high birth weight, which can be resolved through genetically controlled breeding programs (Druet *et al.*, 2014).

2.7 The impact of double muscling on reproduction, carcass, and growth traits

Muscular hypertrophy, similar to some other genetic defects such as syndactylism and osteopetrosis, is associated with both beneficial and adverse consequences in cattle (Ciepłoch *et al.*, 2017; Gebreselassie *et al.*, 2020). However, the challenges that are correlated with the double muscling condition have historically dominated the controversy between breeders over whether to breed for or against the double muscled phenotype (Arnold *et al.*, 2001). Thus, it will be advantageous to cattle breeders to be able to differentiate between beneficial and adverse effects of genetic variants. Table 2.8 summarises the impact of *MSTN* variants on various traits of economic importance. These have been generally reported in literature even though some researchers found different results in other breeds. The effect of the *MSTN* variants on reproduction, growth and carcass traits have been studied extensively. However, the comparisons of the effect of the *MSTN* disruptive variants are challenging across different studies due to different units' expression as other studies uses gross value, percentage of means, and/or proportion of phenotypic SD.

Table 2.8 Summary of studies that investigated the impact of *MSTN* in various cattle breed

Traits affected	Breeds	References
Reproduction		
Reduced fertility	Belgian Blue & Piedmontese	Arthur (1995)
Increased dystocia	Belgian Blue & Piedmontese	Fiems (2012)
Increased calving difficulties	Belgian Blue & Piedmontese	Arthur (1995)
Reduced milk production	Belgian Blue & Piedmontese	Arthur (1995)
Carcass quality		
Increased dressing %	Belgian Blue & Piedmontese	Arthur (1995)
Increased eye muscle area	Angus × Hereford cross	O'Rourke *et al.* (2009)
Increased meat content	Belgian Blue & Piedmontese	Fiems (2012)
Decreased marbling score	Belgian Blue & Piedmontese	De Smet (2004)
Decreased fat content	Belgian Blue[1] , Piedmontese[1]	Allais *et al.* (2010)[2]
	Limousin[2], Charolais	Webb and Casey (2010)
Decreased Organ weight	Belgian Blue & Piedmontese	De Smet (2004)
Decreased carcass bone	Belgian Blue & Piedmontese	De Smet (2004)
Decreased fat depth	South Devon	Wiener *et al.* (2002)
Meat quality		

Traits affected	Breeds	References
Increased protein content	Belgian Blue & Piedmontese	Fiems (2012)
Increased unsaturated fatty acid	Belgian Blue & Piedmontese	Fiems (2012)
Increased lean meat	BelgianBlue[1], Piedmontese[1]	Allais *et al.* (2010)[2]
	Limousin[2], Charolais[2]	Webb and Casey (2010)[1]
Increased muscle fibre hyperplasia	Belgian Blue & Piedmontese	De Smet (2004)
Increased colour lightness	Belgian Blue & Piedmontese	De Smet (2004)
Decreased myoglobin content, oxidative metabolism	Belgian Blue & Piedmontese	De Smet (2004)
Decreased connective-tissue content	Belgian Blue & Piedmontese	De Smet (2004)
Increased Tenderness (high connective-tissue content muscles)	Belgian Blue & Piedmontese	De Smet (2004)
Increased Tenderness (low connective-tissue content	Belgian Blue & Piedmontese	De Smet (2004)
Decreased juiciness and flavour intensity	Belgian Blue & Piedmontese	De Smet (2004)
Increased lean meat yield	Belgian Blue & Piedmontese	Webb and Casey (2010)
Decreased fat content	Belgian Blue & Piedmontese	Webb and Casey (2010); Fiems (2012)
Growth		
Increased birth weight	Belgian Blue & Piedmontese	Arthur (1995); Wiener *et al.* (2009)
Decreased daily weight gain	Belgian Blue & Piedmontese	De Smet (2004)
Decreased intake capacity	Belgian Blue & Piedmontese	De Smet (2004)
Increased feed conversion efficiency	Belgian Blue & Piedmontese	De Smet (2004)
Decreased feed conversion ratio	Belgian Blue & Piedmontese	Arthur (1995)
Increased pre-weaning weight	Belgian Blue & Piedmontese	Flynn and Flynn (2015)
Increased muscle mass	Belgian Blue[1,2], Piedmontese[1]	Arthur (1995)[1]; McPherron and Lee (1997)[1] Druet *et al.* (2014)[2]
Delayed puberty	Belgian Blue & Piedmontese	Flynn and Flynn (2015)
Decreased post -weaning growth rate	Belgian Blue & Piedmontese	Flynn and Flynn (2015)

2.7.1 Reproductive traits

Although incidences of reproduction dysfunction have not been reported to the same extent in other species such as dogs, the well-documented reproductive difficulties are likely the most challenges associated with the double muscling phenotype (Arnold *et al.*, 2001). In cattle, delayed puberty, delayed reproductive development, reduced fertility coupled with high incidences of dystocia are the major challenges that breeders face. Reproduction efficiency of double-muscled cattle is low in females as only 25% fall pregnant after one oestrus while 47% are pregnant at the end of the breeding season (Chupin, 1982). It has been generally reported that cattle that are homozygous for double muscling syndrome are lower in fertility than normal cattle (Arthur, 1995; De Smet, 2004; Bellinge *et al.*, 2005; Fiems and Ampe, 2015) in various cattle breeds. The most common variant that was reported to cause reduction in fertility is the Nt821 variant in Belgian Blue, C313Y in Piedmontese and E291X in Marchigiana breed.

There are several factors that have been reported to be the cause of reduced fertility in homozygous affected animals such as poor sexual behaviour particularly at a young age, calving difficulty, and delayed puberty in both males and females (De Smet, 2004). Sexual behaviour in double-muscled animals is less distinct, making oestrus detection more difficult, especially when using artificial insemination (Farstad, 2018). However, the physiological characteristics of oestrus and ovarian activity in double-muscled cattle does not differ from that of homozygous normal cattle (De Smet, 2004). Reduced sexual odour and small scrotal circumference (SC) which resulted in delayed puberty in homozygous affected males were also reported in literature (Bellinge *et al.*, 2005; Hoflack *et al.*, 2006). Additionally, mature homozygous affected males were found to produce less semen compared to the homozygous normal males; however, there was no reduction in the number of spermatozoa (Arthur, 1995).

Female cattle of the Belgian Blue breed that had the double muscling condition were observed to be less able to carry their calves to term (Bellinge *et al.*, 2005). This resulted in high frequency of dystocia and in most cases these animals undergo caesarean surgery. It is indicated that 82% (Murray *et al.*, 1999) to 90% (Hanzen *et al.*, 1994; Tuska *et al.*, 2021) of Belgian Blue calves are delivered via caesarean surgery. In several European countries, assistance with calving and, finally, caesarean section has become mandatory. The disadvantage of using caesarean section during the calf delivery in double-muscled cows is the significant decrease in pregnancy rate (Fiems, 2012) which causes longer calving intervals (Fiems *et al.*, 2006) and adverse impact on colostrum production (Tuska *et al.*, 2021), extra costs in terms of feeding, housing, labour, poor appetite, uterine infection, lower conception rate, cost of medicine and veterinary treatments (Widyas *et al.*, 2018). The highest frequency of dystocia was observed during the reciprocal crossbreeding of double-muscled dams and sires but the dystocia frequency of mating of double-muscled sires to normal dams was not different from the mating of normal sires and dams (Parkinson *et al.*, 2019).

Reduced pelvic area, a smaller pelvic opening and larger calves at birth are the causes of dystocia and increased perinatal mortality in double muscled animals (Fiems, 2012; Fiems and Ampe, 2015). A review done by Fiems (2012) revealed that double-muscled dams in Charolais and crossbred cows with Q204X variant in their *MSTN* genes had shorter pelvic openings compared to their normal counterparts. However, double-muscled cattle carrying the *MSTN*-F94L genetic variant were reported not to have any negative reproduction performance issues (Sellick *et al.*, 2007; Abe *et al.*, 2009; Lines *et al.*, 2009; Lee *et al.*, 2019) such as reduced fertility, delayed sexual maturation, reduced reproductive fitness, poor viability of offspring (Dilger *et al.*, 2022) or increased dystocia (Short *et al.*, 2002). This might be due to the moderate increase in muscling compared to other breeds (Sellick *et al.*, 2007) such as Belgian Blue and Piedmontese breeds.

The poor viability of the double-muscled calves resulted from poor adaptability of double-muscled calves and poor maternal performance of double-muscled dams (Arthur, 1995). The double-muscled calves are weaker at birth with relatively high incidence of deformities such as enlarged tongues and rachitism (Arthur, 1995). Genetic defects such as double muscling can results in the dam giving birth to abnormal or stillborn calves which add unnecessary trauma to the dam and loss of production for the farmer (van Marle-Köster and Visser, 2021). Furthermore, reducing birth complications reduces not only the cost but also the chances of losing either the calf or the mother (Bellinge *et al.*, 2005). Double muscling resulted in hypoplasia of female and male reproductive systems (Fiems, 2012) and bulls often develop hypogonadism (Patel and Amthor, 2005).

There are some indications in the literature that milk production in double-muscled Belgian Blue and Limousin cattle is 15-30% lower compared to normal-muscled cows (Fiems *et al.*, 2020). The Q204X and F94L variants were reported to be responsible for reducing milk production in double-muscled animals. In cattle breeds where milk production is naturally low, the condition becomes worse for a double-muscled dam to a point where the dam cannot suckle her calf adequately (Arthur, 1995; Fiems and Ampe, 2015). Furthermore, calves are frequently artificially reared to minimize interval-calving period (ICP) of the homozygous affected animals (Fiems *et al.*, 2008). Maintaining a short ICP is a key goal for maximizing calf production and reducing calf mortality (Fiems and Ampe, 2015). However, in certain environments, managing the ICP is more critical than any gene that impacts the phenotypic manifestation of ICP. Having a shorter ICP is beneficial to farmers with the objective of getting a calf from each cow every year. In double-muscled Belgian Blue cows, ICP tends to be longer than in other breeds (Fiems and Ampe, 2015) and compared to homozygous normal cattle (De Smet, 2004). In comparison, suckled double-muscled Belgian Blue cows had an average ICP of 435 days while Blue–Grey, Angus, Simmental, Salers, Limousin, and Hereford cows had an average ICP of 364, 370, 377, 387, 381 and 393 days, respectively. A good

indicator of cattle reproduction efficiency is the ICP (Boligon *et al.*, 2016). The ICP of the double-muscled cattle indicate a poor reproduction efficiency.

2.7.2 Growth traits

MSTN gene is a potential candidate gene for muscle growth as it is responsible for developing muscles in animals (Zhang *et al.*, 2013). Additionally, the rate of muscle growth is positively correlated to daily gain, carcass meat percentage and gain to feed ratio, as it is a determinant of performance (Oksbjerg *et al.*, 2004). Due to the positive genetic association that exists between the different stages, a heavier weaning weight will cause a heavier birth weight, which will cause an overall heavier mature weight (Santana *et al.*, 2014). Double muscling has resulted in the modification of the growth curve in cattle, that is, a higher birth weight resulted from the double muscling phenotype (Ménissier, 1982). In research, double-muscled cattle compared to normal cattle have higher birth weights due to longer gestation periods (Wiener *et al.*, 2002). The birth weight of the double-muscled calves could be as high as 10 to 30% higher at birth than the birth weight of the homozygous normal calves (Jackson *et al.*, 1997). The birth weight of the homozygous affected animals was 13% higher in Blonde d'Aquitaine (Vinet *et al.*, 2021), 12% higher in Piedmontese calves (Short *et al.*, 2002) and South Devon calves (Wiener *et al.*, 2009) than the birth weight of the homozygous normal calves for T3811>G3811, CY31Y, and Nt821 variant, respectively. This was within the lower range of 10-30% proposed firstly by Ménissier (1982). A negative correlation between birth weight and pelvic area in cattle was discovered to be the cause of the dystocia (Bellows *et al.*, 1971).

Linearly increased birth weight and linearly decreased pelvic area in homozygous affected animals were confirmed to be the cause of high incidence of dystocia and the reason for systematically applied caesarean section (Short *et al.*, 2002; Coopman *et al.*, 2004). For example, Piedmontese heifers and cows that were homozygous affected for C313Y variant were 49.6% and 7.9 % higher in dystocia score than homozygous normal animals, respectively. (Short *et al.*, 2002). It was suggested that selection for lower birth weight and larger pelvic area in homozygous affected animals might reduce the incidence of dystocia and routine caesarean section (Coopman *et al.*, 2004). There are other genetic variants in the *MSTN* gene such as Q204X, F94L, E226X and E291X that causes an increase in muscularity which results in heavier birth weights in cattle breeds such Charolais (Dunner *et al.*, 2003), Limousin (Casas *et al.*, 2004), Marchigiana (Marchitelli, 2003), and Maine-Anjou (Grobet *et al.*, 1998). Additionally, other genetic variants such as S105C and D182N causes increase in muscularity with no change in birth weights in the same cattle breeds such as Parthenaise and Maine-Anjou (Dunner *et al.*, 2003; Haruna *et al.*, 2020).

Throughout the postnatal period until weaning stage, the higher growth rate of double muscled cattle is evident while it decreases in relation to their normal contemporaries in the post-weaning period (Flynn and Flynn, 2015). The decrease in growth rate during the post-weaning phase results in a lower

mature weight (Arthur, 1995) due to lower feed intake (Flynn and Flynn, 2015). During the weaning phase, some studies reported increased weaning weight (Arthur, 1995; Cafion *et al.*, 2002) in double-muscled animals while other researchers indicated that double-muscled calves are more likely to die pre-weaning (Casas *et al.*, 2004). Weaning weight in double-muscled Limousin cattle with F94L variant was higher (206 kg) than in homozygous normal cattle (202 kg) (Bennett *et al.*, 2019) while in Piedmontese cattle with double muscling syndrome affected by C313Y variant weaning weight was lower (166 kg) than in homozygous normal (174 kg) (Short *et al.*, 2002). Other researchers found higher weaning weights in heterozygous carrier animals compared to the homozygous normal animals. Heterozygous carriers of Nt821 variant in Charolais and Belgian Blue × British Breed were heavier at weaning (253 ± 3 kg and 253 ± 3 kg) than homozygous normal animals (244 ± 1 kg and 228 ± 2 kg) (Casas *et al.*, 2004). Also, Piedmontese cattle that were heterozygous carriers of C313Y variant were 9.1 ± 4 kg higher weaning weight than homozygous normal animals (Casas *et al.*, 1999). 0

Feed conversion efficiency are substantially increased as a result of the combination of comparable average daily weight gain and lower feed intake (De Smet, 2004; Flynn and Flynn, 2015). The improved feed conversion is more likely due to a shift in the composition of body weight gain toward more protein and less fat deposition, rather than improvement in feed digestibility or maintenance requirements (De Smet, 2004). Cattle that are double muscled tend to have locomotion problems when they start gaining weight (De Smet, 2004). This is due to the reduction in bone weight which causes poor rear-leg structure and movement difficulties (Ansay and Hanset, 1979). Bones of the double-muscled animals are more heavily loaded and bone strength is reduced because the reduced skeleton carries the heavy body weight (Fiems, 2012). General factors that regulate the manifestation of the growth potential, such as management, maternal ability of the dam, calf sex, and dietary regime, influence the amount and direction of growth discrepancies between double-muscled and normal cattle (Arthur, 1995). The conformational challenges in the double-muscled cattle are directly caused by higher birth weight and muscle growth pattern in calves and these animals are susceptible to accidental development difficulties, especially of lungs and heart (Fiems, 2012).

2.7.3 Carcass traits based on Real Time Ultrasound measurements

Real-time ultrasound (RTU) is essential for determining the distribution of fat and body composition in the live animals (Araujo, 2003; Seroba *et al.*, 2011; De Vos, 2018). RTU scans are an effective selection tool and non-invasive way to assess beef cattle's body composition (Polák *et al.*, 2007; Drennan *et al.*, 2009; Gupta *et al.*, 2013). The three points on an animal where the ultrasound measurements are collected are shown in Figure 2.8. Point 1 is the marbling (percentage intramuscular fat), point 2 is the

eye muscle area (EMA) and backfat thickness and point 3 is the rump fat thickness (Silcox, 2005; Hicks, 2011).

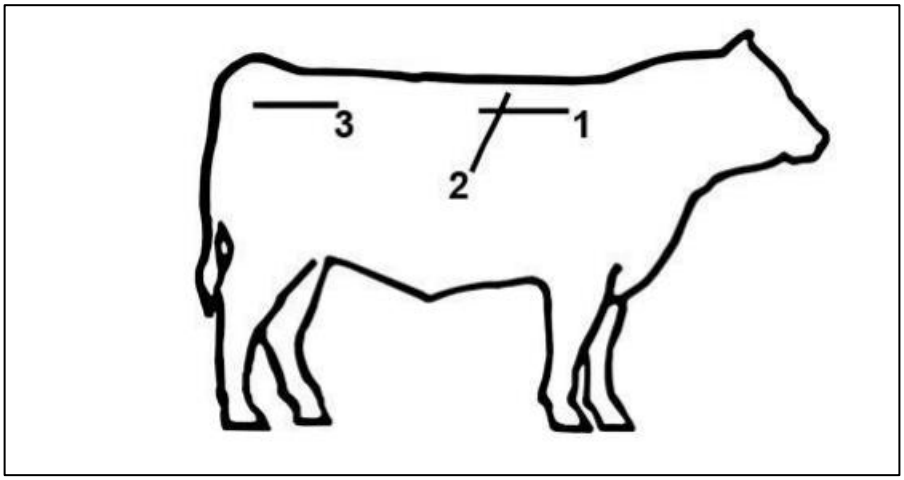

Figure 2.8 Graphic presentation showing the three points where RTU measurements are taken (Hicks, 2011)

The beef market relies heavily on knowledge about carcass and meat quality, and many cattle production systems' future depend on it (Ceccobelli *et al.*, 2022). A key component of profitability in beef production is the meat yield. It is critical to gain a better understanding of the effects of contributing genes in beef cattle breeds in order to explain variability in carcass and meat quality traits (Sarti *et al.*, 2019).

Research brought to light the effect of *MSTN* variants on carcass and meat quality in heterozygous carrier and double-muscled animals. The greatest merit that defines the double muscling syndrome in cattle is the superior carcass characteristics (De Smet, 2004). This resulted from muscular hypertrophy, fineness of bones, lower potential to accumulate fat and smaller digestive tract of the double-muscled cattle (Arthur *et al.*, 1989; Oliván *et al.*, 2004). Different *MSTN* variants showed to significantly affect different carcass traits in different cattle breeds. Angus-sired cattle that were heterozygous carriers of *MSTN* 821 *del11* (Nt821) variant were heavier at slaughter, with higher conformation class scores and heavier hindquarters, they had heavier sirloins, both before and after maturation and larger eye muscle area, resulting in general muscle mass increase for the heterozygous carrier animals. Similar results were obtained in heterozygous carriers of Nt821 variants in South Devon (Wiener *et al.*, 2002), Angus × Hereford and Angus × Charolaise crosses (O'Rourke *et al.*, 2009) and, Belgian Blue and Piedmontese (Casas *et al.*, 1998). In terms of EMA, a study found no significant difference between homozygous normal (80.31 ± 2.29 cm^2), heterozygous carrier (82.00 ± 0.64 cm^2) and homozygous affected (83.15 ± 0.33 cm^2) Hanwoo steers for *MSTN* g.2371T>A (Han *et al.*, 2012). In contrast, O'Rourke *et al.* (2009) reported a significant difference in EMA of homozygous normal (70.40 cm^2) and homozygous affected (85.00 cm^2) Angus × Hereford cross with the *MSTN* 821 *del11*.

There has been some indication in earlier reports that animals with double muscling syndrome produced tough and dark-cutting meat with poor quality (Culley, 1807; MacKellar, 1960). This was later reviewed by Clinquart *et al.* (1998) in Belgian Blue cattle where meat toughness was reported to be increased in double-muscled animals of this breed. Fasting for 48 hours caused double muscled cattle to have a much higher pH before slaughter, which caused some animals to exhibit dark cutting (Fiems, 2012). Most of the studies focused on the longissimus muscles of cattle that were homozygous for double muscling condition. A review done by Arthur (1995) on longissimus muscles samples indicated that meat from animals with two copies of mutant allele in the *MSTN* gene was significantly more tender than those with no mutant allele. Several researchers (Webb and Casey, 2010; Boukha *et al.*, 2011; Chelh *et al.*, 2011; Moczkowska *et al.*, 2015) found similar effects of the Nt821 variant in Belgian Blue cattle where meat from double-muscled animals were more tender than in homozygous normal cattle. In backcross Limousin × Jersey cattle, F94L-homozygous affected animals had 9.8% more tender in semitendinosus meat than animals with homozygous normal genotype (Lines *et al.*, 2009). This can be attributed to reduced collagen content, reduced proportion of stable non-reducible cross links and smaller muscle fibres in double-muscled animals compared to homozygous normal animals (Bailey *et al.*, 1982; Boukha *et al.*, 2011; Chelh *et al.*, 2011). Also, heterozygous carriers of C313Y variant in the Piedmontese-cross cattle (Wheeler *et al.*, 2001) and heterozygous carrier of Nt821variant in Belgian Blue cattle (Arthur, 1995) were reported to be significantly more tender than homozygous normal animals. Not every study has discovered a correlation between double muscling and increased tenderness (Uytterhaegen *et al.*, 1994). Studies done with Asturiana de los Valles breed (Oliván *et al.*, 2004), Angus-sired breed (Gill *et al.*, 2009) and South Devon breed (Wiener *et al.*, 2009) found no significant correlation between *MSTN* variants of interest and meat tenderness.

There are several types of fats in animals such as subcutaneous and intramuscular fat. Subcutaneous fat which is the layer between the skin and muscles includes backfat thickness and rump fat thickness. The effect of the *MSTN* variants on the subcutaneous and intramuscular fat is well documented in research. Wiener *et al.* (2002) found that *MSTN* 821 *del11* decreased fat in double-muscled South Devon cattle (23.80 ± 2.29 mm) compared to homozygous normal animals (32.40 ± 1.26 mm). Similar observation was made in Marchigiana beef cattle where heterozygous carrier bulls of E291X variant showed significant lower fat content (6.62%) than normal bulls (10.37%) (Ceccobelli *et al.*, 2022). In Korean cattle, *MSTN* g.-371T>A had no effect on fat, resulting in no significant difference between homozygous normal (12.69 ± 1.26 mm), heterozygous carrier (12.53 ± 0.35 mm), and homozygous affected cattle (12.18 ± 0.18 mm). A *MSTN*-F94L variant in Limousin × Jersey backcross (Esmailizadeh *et al.*, 2008) and in F2 Limousin x Japanese Black cattle (Abe *et al.*, 2009) decreased fat depth by -18.7%, and total carcass fat weight by -16.5% in homozygous affected animals compared to the normal cattle. The rate of fat deposition in double-muscled

cattle was slower than in homozygous normal animals (Casas *et al.*, 2004; Esmailizadeh *et al.*, 2008; Allais *et al.*, 2010; Alemneh and Getabalew, 2019; Bennett *et al.*, 2019) resulting in delayed slaughter age.

Intramuscular fat, often known as marbling, is the last fat depot found between muscle cells in animals (Komolka *et al.*, 2014; Silva *et al.*, 2015; Moloney and McGee, 2017). While some studies found no influence of the double muscling condition on carcass marbling, some have shown lower marbling in double-muscled cattle. Limousin cattle that were homozygous affected by F94L variant had lower marbling score (29.4%) than those that were homozygous normal (34.5%) (Bennett *et al.*, 2019). In addition, Esmailizadeh *et al.* (2008) and Abe *et al.* (2009) found that mutant allele of *MSTN*-F94L variant reduced intramuscular fat by -8.2% in Limousin × Jersey and F2 Limousin x Japanese Black cross cattle, respectively. These results were similar to the observations made in Belgian Blue × British breed genotyped for Nt821 variant (Casas *et al.*, 2004). Homozygous affected showed lower marbling score (380 ± 18, USDA yield), compared to heterozygous carrier (498 ± 6, USDA yield) and homozygous normal cattle (549 ± 5, USDA yield) (Casas *et al.*, 2004). On the contrary, Han *et al.* (2012) found no statistically significant differences between the marbling score of homozygous normal (7.00 ± 0.51 mm), heterozygous carrier (6.18 ± 0.14 mm) and homozygous affected (6.13 ± 0.07 mm) Hanwoo steers genotyped for *MSTN* g.2371T>A. Furthermore, heterozygous carrier of *MSTN* 821 *del11* in Angus × Hereford cows were also lower in marbling score (3.1 ± 0.4 mm) than the homozygous normal cows (4.0 ± 0.1 mm) (O'Rourke *et al.*, 2009). However, in Angus × Hereford calves, heterozygous carriers of *MSTN* 821 *del11* variants were not statistically different from the homozygous normal calves (O'Rourke *et al.*, 2009). Italian consumer preferred meat from the Piedmontese cattle because of reduced intramuscular fat content (Renna *et al.*, 2019). The degree of marbling affects juiciness and meat flavour in the sense that decreased marbling corresponds to a lower juiciness and meat flavour grade (Muchenje *et al.*, 2009). Reduced marbling in double-muscled cattle is due to decreased subcutaneous and internal fatty tissue adipocyte size (Bellinge *et al.*, 2005).

The genetic variants in the *MSTN* gene prevents fat accumulation, resulting in extremely lean meat of double-muscled cattle (Hope *et al.*, 2013; Casas and Kehrli Jr, 2016; Farstad, 2018). The combination of higher lean-meat content in carcass and lower fat and bone content and smaller internal organs result in extremely high dressing percentage in double-muscled animals (De Smet, 2004; Webb and Casey, 2010). The carcass from double-muscled animals was generally reported to dresses out at 60-70% (Lasagna *et al.*, 2005) which is 19% higher than those of animals that doesn't exhibit double muscling syndrome. This resulted from a decreased digestive tract, lower skin weight and organs (De Smet, 2004). In the study done by Raes *et al.* (2001), homozygous affected and heterozygous carriers of Nt821 variant in Belgian Blue bulls reached 659 g/kg and 638 g/kg in dressing yield, while 602 g/kg was observed in homozygous normal bulls at the *MSTN* gene. All these three genotypes differed significantly. The dressing percentage observed

in purebred Angus and Charolaise breed was greater in heterozygous carrier (59.6%) of Nt821 variant than in homozygous normal cattle (57.3%) (O'Rourke *et al.*, 2009). Although no statistical significant difference was observed, heterozygous carriers of E291X variant in Marchigiana bulls had higher in dressing yields (62.65%) than homozygous normal bulls (60.96%) (Ceccobelli *et al.*, 2022).

The muscle hypotrophy, and the fat and bone hypotrophy, are general but not uniform throughout the body (De Smet, 2004). The limb bones are reduced and thinner in double-muscled cattle (Bellinge *et al.*, 2005). Superficial muscles and the hind limbs are the most affected parts in double-muscled cattle compared to the fore limbs, although there are variations between studies in terms of relative muscle hypertrophy that has been reported (De Smet, 2004). The limbs' bones are shorter and thinner following the same gradients observed for the muscles (De Smet, 2004). Double-muscled cattle have been reported to have up to 30% higher muscle : bone ratio than normal cattle (Fiems *et al.*, 2020). At the level of the shoulders and thighs, where muscular hypertrophy is often most evident, the muscle-to-bone ratio is at its highest (De Smet, 2004).

2.8 Conclusion

The genetic variants that disrupt *MSTN* function influence muscle development and have an impact on economically important traits. As a result, it's critical for breeders and farmers to investigate *MSTN* gene variants, as this can help them improve selection strategies and performance. While double-muscled animals produce more meat and have other advantages, the double muscling syndrome comes with several drawbacks. This raises questions regarding the suitability of double-muscled cattle in the conditions prevalent in South Africa. The widespread occurrence of the *MTSN* gene in South Africa carrier animals is yet to be determined, even though it is present in some South African breeds and herds.

Chapter 3 Materials and Methods

3.1 Introduction

This study investigated the prevalence of myostatin variants in SA beef breeds and the association of these variants with animal performance. The respective breeders' Societies provided consent for the study and the University of Pretoria Ethics Committee (Natural and Agricultural Science) granted permission to use the external data set (NAS223/2020). The project was conducted in two phases, with phase one analysing genotypic data from the pilot study and phase two using phenotypic and genotypic data.

3.2 Materials

3.2.1 Phase 1: Pilot study

The Beef genomic program (BGP) was established in 2015 with the aim to build reference populations for SA beef breeds for application in genomic selection (Walsh and Spazzoli, 2018). BGP generated genotypes over a three-year period and included a pilot study to investigate the prevalence of double muscling variants in the national beef populations. Five South African beef cattle breed societies namely Bonsmara, Beefmaster, Brangus, Drakensberger and Limousin were included in the pilot study.

For the pilot study, farmers selected animals that were phenotypically well muscled and suspected of being *MSTN* carriers. Hair samples were collected by farmers and these were submitted for genotyping via BGP Association using the International Dairy Beef (IDBv3) SNP chip (Mullen *et al.*, 2013) at Weatherbys in Ireland. This array features 53 714 SNP probes distributed across the whole bovine genome with an average spacing of 37.4 kb as well as nine *MSTN* SNP variants within the *MSTN* gene located on BTA 2. The following nine *MSTN* variants are routinely included for diagnostic testing of myostatin namely, Nt821, Q204X, F94L, E226X (E291X), C313Y (Nt419), S105C (D182N), Nt414 (Nt748), L64P and Nt267 (Nt324). The different breeds, and numbers of animals per breed genotyped in phase one are summarised in Table 3.1.

Table 3.1 Summary of breeds and the number of animals genotyped in phase one (pilot study).

Breed	Population Size (n)
Bonsmara	83
Beefmaster	46
Brangus	54
Drakensberger	97
Limousin	75
Total	**355**

3.2.2 Phase two

3.2.2.1 Genotypic data

For phase two of the project, two of the Breed Societies namely the SA Bonsmara and the Drakensberger Society continued with voluntary screening for suspected carrier animals of myostatin and submitted hair samples to SA Stud Book Association for genotyping. Animal were genotyped over a three (Bonsmara) and four (Drakensberger) year period. All samples were genotyped using the IDBv3 SNP array as for the pilot study (Phase one). The myostatin test results received was coded with 0, 1 and/or 2; with 0 indicating that the animal was homozygous for the normal alleles at the specific locus, 1 indicating that the animal was a heterozygous carrier of the specific mutation; and 2 indicating that the animal was homozygous for the double muscled (affected) mutation at the specific locus. Table 3.2 provides a summary of the data available for analyses based on the number of animals tested per year and the test result for the genetic variants. The number of variants included on the SNP array in this study differed.

Table 3.2 Summary of number of animals recorded based on the diagnostic test available for the different variants over a period of 3 and 4 years for Bonsmara and Drakensberger cattle respectively

			Years			
Breeds	**Number of animals genotyped**	**Myostatin variants**	**2018**	**2019**	**2020**	**2021**
Bonsmara	1378	Nt748	226	653	499	-
	1260	Nt414	108	653	499	-
	1418	Nt267	266	653	499	-
	1778	Q204X	622	656	500	-
	1778	Nt821	622	656	500	-
	1778	F94L	622	656	500	-
Drakensberger	308	Nt748	5	97	181	25
	303	Nt414	-	97	181	25
	388	Nt821	84	98	181	25
	308	Nt267	5	97	181	25

In Table 3.3 the distribution of females versus males genotyped for the myostatin variant is shown.

Table 3.3 Distribution of females and males based on the different myostatin variants

Breeds	Myostatin variants	Females	Males
Bonsmara	Nt748	338	1040
	Nt414	368	892
	Nt267	376	1042
	Q204X	627	1151
	Nt821	627	1151
	F94L	627	1151
Drakensberger	Nt748	36	272
	Nt414	36	267
	Nt821	89	299
	Nt267	36	272

Missing genotypes were observed among the genetic variants in both the Bonsmara and Drakensberger breeds after processing of the prepared data. The number of Bonsmara and Drakensberger animals with missing data records is shown in Table 3.4.

Table 3.4 Percentages of missing data records of the Bonsmara and Drakensberger animals

Percentage of missing data records	Bonsmara	Drakensberger
Number of data records	1778	388
Number of missing data records		
Nt748	400 (22.50%)	80 (20.62%)
Nt414	518 (29.13%)	85 (21.91%)
Nt267	360 (20.25%)	80 (20.62%)
Q204X	0 (0.00%)	-
Nt821	0 (0.00%)	0 (0.0%)
F94L	0 (0.00%)	-

3.2.2.2 Phenotypic data

For phase two, genomic estimated breeding values (GEBVs) which are part of routine genetic evaluations for the SA Bonsmara and Drakensberger breeds, were included for all the animals that were genotyped. The GEBV data set included twelve (12) traits namely: age at first calving (AFC), inter-calving period (ICP), scrotal circumference (SC), longevity, direct birth weight (BW_{DIR}), direct weaning weight

(WW_{DIR}), average daily gain (ADG), feed conversion ratio (FCR), fat, marbling, and eye muscle area (EMA) (Table 3.5).

Table 3.5 Brief description of the traits for SA Bonsmara and Drakensberger breeds analysed in the study (SA Stud Book, 2016)

Category	Trait	Description (units of measurements)
Reproduction	Age at first calving (AFC)	Age at first calving is measured as number of days between birth and first calving (months).
	Inter-calving period (ICP)	The period between the birth of one calf and the birth of the next calf in a cow's life. The inter-calving cycle is one of the most critical criteria for assessing a farm and/or population's productivity and reproductive performance (days).
	Scrotal circumference (SC)	Scrotal circumference is measured when bulls are weighed between 365 to 540 days and is used to estimate the consistency and quantity of spermatozoa-producing tissue as well as age at puberty (mm).
	Longevity	Female longevity refers to how long a female stays in the breeding herd after giving birth, and it is affected by many economically important traits including female reproduction (months).
Growth	Direct birth weight (BW_{DIR})	Birth weight is used as a selection criterion to increase calving ease and is an effective measure of animal viability (kg).
	Direct Weaning weight (WW_{DIR})	In cow–calf systems, weaning weight is a good indicator of production. Weaning weights are measured for each calf between 160 and 250 days of age (kg).
	Average daily gain (ADG)	In the beef cattle industry, ADG is a significant trait that contributes to production quality and economic benefits (g).
	Feed conversion ratio (FCR)	FCR is the ratio of dry matter intake to live-weight gain, and it's a good way to track or describe feedlot cattle performance efficiency (kg/kg). FCR is measured in growth tests where individual intake is also recorded.
Carcass (RTU measurements)	Backfat thickness	Measured as the subcutaneous fat layer over the longissimus dorsi muscles between the 12^{th} and the 13^{th} rib (mm).
	Rump fat thickness	Measured at the junction of the biceps femoris and gluteus medium between the hook and pin bones. This is more commonly known as subcutaneous fat depth at the P8 site (mm).

Category	Trait	Description (units of measurements)
	Eye Muscle area (EMA)	The region of the eye muscle is a useful measurement for predicting meat yield and the amount of external fat. It is determined in the area of the longissimus thoracicus et lumborum. The eye muscle region predicts the lean-to-fat ratio (cm^2).
	Marbling	The percentage of intramuscular fat and has a positive effect on sensory quality traits such as taste, juiciness, and tenderness of meat in beef producing cattle (mm).

mm: millimetre,
kg: kilogram,
cm^2: centimetre squared

3.3 Methods

3.3.1 Phase 1: Pilot study

The genotypic *MSTN* variant data was received from SA Stud Book. The data was edited before statistical analysis, to remove duplicate animals and animals with no identification numbers. The frequencies (genotype and allele) of the various *MSTN* variants identified per breed was estimated using Microsoft Office Excel (Microsoft, 2016). The mathematical model for genotype and allele frequency (Nei and Kumar, 2000) is as follows:

Genotype frequency = Xi = $\frac{Gi}{N} \times 100\%$

Allele frequency = Xi = $\frac{2nii+2nij}{2N}$

Where:

Xi = Genotype or allele frequency,

i th = homozygous alleles,

j th = heterozygous alleles,

Gi = number samples of i genotype

N = total samples

3.3.2 Phase two

3.3.2.1 Data analysis

Data on genotypic *MSTN* variants and GEBVs of traits of interest for SA Bonsmara and Drakensberger breeds were provided. All genetic variants with genotypes were evaluated, and animals with missing ID, as well as duplicates, were removed from the raw data. The data set provided consisted of 13 genetic variants in total. The data set was analysed to remove all the animals which were homozygous normal for all the 13 genetic variants. The affected SA Bonsmara animals had four genetic variants, and the Drakensberger animals had three genetic variants present within the study populations. Genotypic

frequencies for SA Bonsmara and Drakensberger animals per genetic variant as described under 3.3.1 for Phase one were calculated.

Listwise deletion method was used to eliminate missing data records. The generated data set was imported into IBM Statistical Package for Social Sciences (SPSS) version 27, where descriptive statistical analysis parameters were estimated. The software's settings were aligned to the data set that was analysed by classifying variable types, data types, and data labels, as well as validating that the software was configured to a standard confidence level of 95%.

3.3.2.2 Statistical analysis

Statistical analysis was conducted using SPSS software (version 27; IBM) and reported as mean ± standard error (SE). An independent t-test was used to identify the statistical differences between groups of animals. The normality of the data was considered using the Shapiro–Wilk test with Levine test used to determine equal variances, and log transformations (log10) were performed where normal distribution was violated. Prior to completing the multivariate analysis of variance (MANOVA) for the reproductive, carcass, and growth traits, it was necessary to test the assumption that there is no collinearity among the dependent variables; thus, a set of Pearson correlations between all of the dependent variables were performed and all the correlation coefficients were less than 0.80.

Data which included traits and genotypes were statistically analysed by means of two-way MANOVA using the General Linear Models (GLM) procedure of SPSS version 27 (Pallant, 2020). The following fundamental equation for GLM model for the effects of genotypes of different SNPs on traits of interest was used for analysis:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots. \beta_k X_k + E \qquad (1)$$

Where

α is the constant value

β is the beta coefficients

X is the independent variables (factors)

E is the error term

The Bonferroni multiple range test was applied in most cases since the data was imbalanced. Nt748, Nt414, Nt267, Q204X variables for Bonsmara animals, and Nt748, Nt414, and Nt821 variables for Drakensberger animals were modelled as fixed factors and BW_{DIR}, WW_{DIR}, ADG, FCR, AFC, ICP, SC, longevity, Fat, Marbling and EMA variables were modelled as covariates. Differences between means were tested by means of the Least Significant Difference (LSD) multiple range test. The statistical differences were considered significant at a probability level of 5% ($p < 0.05$).

For the animals that had more than one variant in their *MSTN* gene, combinations codes were generated to test for their effects on different traits of interest in this study. Cumulative effects of the *MSTN* variants on different traits were estimated for the SA Bonsmara and Drakensberger populations using the SPSS procedure version 27, with the same model as in equation 1. Dunnett's test from GLM procedure was used to estimate mean differences. Dunnett t-tests treat one group as a control and compare all other groups against it. The combination 0000 for Bonsmara and 000 for Drakensberger were treated as control. Significant difference was considered at a $p < 0.05$. The mean and the standard error (SE) of traits assessed in relation to a combination of four genetic variants in SA Bonsmara and three genetic variants in the Drakensberger population were described using descriptive statistics. For Bonsmara, combinations that had less than four valid values were excluded and for Drakensberger, combinations that had less than three valid values were excluded. The combinations for the Bonsmara are in the following order, Nt748, Nt414, Nt267 and Q204X, and Nt748, Nt414 and Nt821 for Drakensberger animals. The combinations of four genetic variants observed in SA Bonsmara animals are summarised in Table 3.6.

Table 3.6 The combinations of the existing genetic variants detected in the SA Bonsmara animals

Genetic variants				
Nt748	**Nt414**	**Nt267**	**Q204X**	**Combinations**
0	0	0	0	0000
1	0	1	0	1010
1	1	0	0	1100
1	1	0	1	1101
2	0	1	0	2010
2	0	2	0	2020
2	1	0	0	2100
2	1	0	1	2101
2	1	1	0	2110
2	1	1	1	2111
2	2	0	0	2200
2	2	0	1	2201

The combinations of three genetic variants observed in Drakensberger animals are summarised in Table 3.7.

Table 3.7 The combinations of the existing genetic variants detected in the Drakensberger animals

Genetic variants			
Nt748	**Nt414**	**Nt821**	**Combinations**
0	0	0	000
1	0	1	101
1	1	0	110
1	1	1	111
2	1	0	210
2	2	0	220

Figure 3.1 is a flow diagram that depicts the steps that were followed for data and statistical analyses for Phase 2.

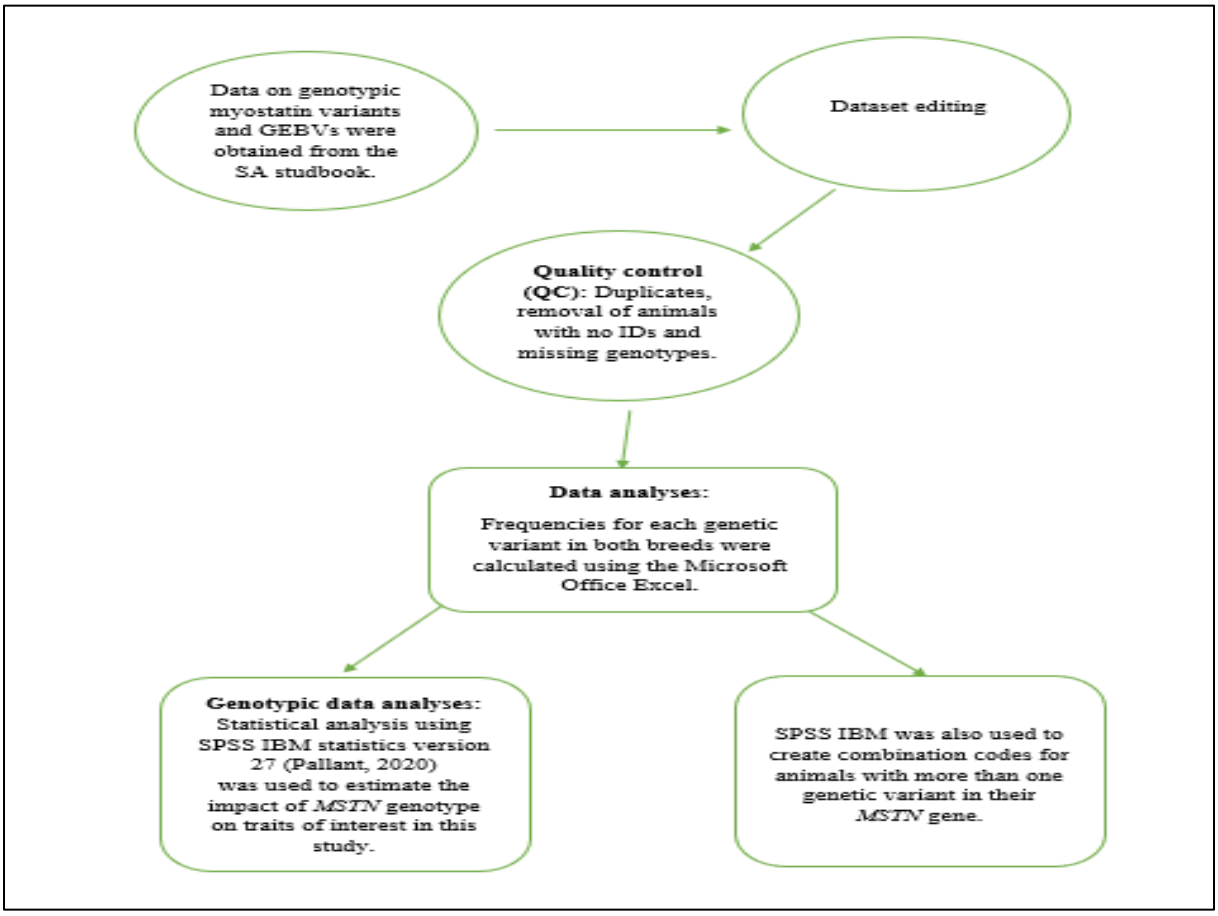

Figure 3.1 Flow diagram demonstrating the series of steps followed for statistical analysis

Chapter 4 Results

4.1 Phase 1

4.1.1 Genotypic frequencies

In Phase one, only three (Nt821, F94L and Q204X) of the nine known *MSTN* variants present on the IDB v3 SNP array were observed in the 355 animals genotyped in the Bonsmara, Beefmaster, Brangus, Drakensberger, and Limousin breeds. Across breeds, 21.13% of the homozygous affected animals presented with the F94L variant, while the majority of heterozygous animals (27.04%) carried the Nt821 variant (Table 4.1).

Table 4.1 Genotypic frequencies of *MSTN* variants across 355 animals in Phase 1

Myostatin Variants	Genotypic Frequency		
	Homozygous normal (%)	**Heterozygous carrier (%)**	**Homozygous affected (%)**
Nt821	225 **(63.37)**	96 **(27.04)**	34 **(9.58)**
F94L	275 **(77.46)**	5 **(1.41)**	75 **(21.13)**
Q204X	299 **(84.22)**	40 **(11.27)**	16 **(4.51)**

The number of animals that were affected by the *MSTN* variants detected per breed and their frequencies are summarised in Table 4.2. The F94L variant was limited to the Limousin and the Brangus breeds. The Nt821 variant was observed in all the heterozygous carriers of all the breeds, except for the Limousin. Frequencies for homozygous affected animals were low, except for the Nt821 in the Beefmaster (0.46%) and Limousin (1.00) for F94L variant. Heterozygous carriers varied from 0.48 in the Bonsmara for Q204X to 0.43, 0.46 and 0.52 for Nt821 in the Brangus, Drakensberger and Beefmaster, respectively.

Table 4.2 Number of animals affected per myostatin variant per breed and their frequencies

Breeds	Population size (n)	Myostatin variants	Homozygous normal (%)	Heterozygous carrier (%)	Homozygous affected (%)
Bonsmara	83	Nt821	79 **(95.00)**	4 **(5.00)**	0 **(0.00)**
		Q204X	27 **(33.00)**	40 **(48.00)**	16 **(19.00)**
Beefmaster	46	Nt821	1 **(2.00)**	24 **(52.00)**	21 **(46.00)**
Brangus	54	Nt821	30 **(56.00)**	23 **(42.00)**	1 (**2.00)**
		F94L	49 **(91.00)**	5 **(9.00)**	0 **(0.00)**
Drakensberger	97	Nt821	40 **(41.00)**	45 **(46.00)**	12 **(13.00)**
Limousin	75	F94L	0 **(0.00)**	0 **(0.00)**	75 **(100.00)**

4.1.2 Allelic frequencies

Allelic frequencies of *MSTN* variants detected within the breeds are indicated in Table 4.3 indicating the highest frequency for F94L (100%) in the Limousin breed while the Beefmaster breed had the highest frequency of Nt821 (72%) followed by the Drakensberger (36%).

Table 4.3 Allelic frequencies of *MSTN* variants reported within the breeds

Breeds	Allelic Frequency		
	Myostatin Variants	**Dominant Allele (p)**	**Recessive Allele (q)**
Bonsmara	Nt821	0.98	0.02
	Q204X	0.57	0.13
Beefmaster	Nt821	0.28	**0.72**
Brangus	Nt821	0.77	0.23
	F94L	0.95	0.05
Drakensberger	Nt821	0.64	**0.36**
Limousin	F94L	0.00	**1.00**

4.2 Phase two

4.2.1 Genotypic frequencies

In phase two, 13 *MSTN* variants were screened in the Bonsmara and Drakensberger breeds. Seven of the thirteen variants (C313Y, D182N, E226X, E291X, Nt419, S105C, Nt324) were not detected in either of the breeds. In the SA Bonsmara Nt748, Nt414, Nt267 and Q204X and in the Drakensberger Nt748,

Nt414, Nt821 were detected and summarised in Table 4.4 and 4.5. Only variants with a frequency > 5% were included for subsequent analysis. In the Bonsmara breed, the variant Nt748 was the most common in both heterozygous carrier (48.82%) and homozygous affected (35.02%) animals, followed by the Nt414 variant in both heterozygous carrier (42.46%) and homozygous affected (8.10%) animals as shown in Table 4.4.

Table 4.4 Genotype frequencies of *MSTN* variants detected in the SA Bonsmara animals

Variants	Number of animals	Genotyped animals		
		Homozygous normal	**Heterozygous carrier**	**Homozygous affected**
Nt748	1362	236	665	477
		(17.33%)	**(48.82%)**	**(35.02%)**
Nt414	1260	623	535	102
		(49.44%)	**(42.46%)**	**(8.10%)**
Nt267	1418	1043	344	32
		(73.55%)	**(24.26%)**	**(2.26%)**
Q204X	1778	1261	496	21
		(70.92%)	**(27.89%)**	**(1.18%)**

Bold indicates the percentage

Table 4.5 indicates the genotypic frequencies of the *MSTN* genetic variants that were reported in the Drakensberger animals. The Nt748 genetic variant showed the highest frequency in homozygous affected (12.01%) and heterozygous carrier (38.96%) animals.

Table 4.5 Genotype frequencies of *MSTN* variants detected in the Drakensberger breed

Variants	Number of animals	Genotyped animals		
		Homozygous normal	**Heterozygous carrier**	**Homozygous affected**
Nt748	308	151	120	37
		(49.03%)	**(38.96%)**	**(12.01%)**
Nt414	303	203	86	14
		(66.99%)	**(28.38%)**	**(4.62%)**
Nt821	388	255	124	9
		(65.72%)	**(31.96%)**	**(2.32%)**

Bold indicates the percentage

4.2.2 Association analysis

4.2.2.1 Bonsmara animals

Prior to completing a series of follow-up ANOVAs, the assumption of homogeneity of variance was tested for all twelve traits. The homogeneity of variance assumption was satisfied for BW_{DIR}, WW_{DIR}, ADG, FCR, EMA, AFC, ICP and longevity since the $p > 0.05$ indicated that the error variances between the groups were equal. Additionally, Levene's test was significant for fat, marbling, and SC ($p < 0.05$).

Table 4.6 summarises the impact that various genetic variants had on reproductive traits in Bonsmara animals. All reproduction traits in this study (AFC, ICP, SC and longevity) were significantly affected by Q204X variant with $p < 0.05$. In this case, heterozygous carrier animals were negatively affected with increased AFC, increased ICP, and decreased SC values while the longevity of the same group was positively affected with increased longevity value compared to homozygous normal animals. Nt748, Nt414 and Nt267 variants in this study had a significant effect ($p < 0.05$) on longevity. Homozygous affected group for Nt414 and Nt267 variants had decreased longevity compared to other groups in these two variants and similar results were also observed in heterozygous carriers of Nt748 variant. There was a significant association between the Nt748 and Nt267 variants and AFC ($p < 0.05$) and all groups of homozygous affected animals had shorter AFC in these variants compared to other groups. The ICP of the Bonsmara animals was significantly affected ($p < 0.05$) by both Nt414 and Nt267 variants and heterozygous carrier and homozygous affected had decreased ICP in Nt414 and Nt267, respectively.

Table 4.6 Descriptive statistics based on GEBVs for reproduction traits in the Bonsmara population included in this study

Genetic variants	Genotypes	N	Reproduction traits			
			AFC (months), Mean ± SE	ICP (days), Mean ± SE	SC (mm), Mean ± SE	Longevity (months), Mean ± SE
Clean animals	0000	209	-8.08 ± 0.67	-2.47 ± 0.19	14.55 ± 0.57	29.34 ± 0.32
Nt748	0	236	-6.23^{a} ± 1.36	-3.07 ± 0.42	12.81 ± 1.22	27.36^{a} ± 0.65
	1	665	-8.30^{b} ± 0.97	-3.03 ± 0.30	12.32 ± 0.87	26.48^{b} ± 0.46
	2	477	-8.27^{b} ± 0.71	-2.89 ± 0.22	12.92 ± 0.64	27.05^{a} ± 0.34
Nt414	0	623	-8.69 ± 0.75	-2.45^{a} ± 0.23	13.21 ± 0.67	28.41^{a} ± 0.36
	1	535	-7.57 ± 0.94	-3.36^{b} ± 0.29	12.73 ± 0.84	27.10^{b} ± 0.45
	2	102	-6.54 ± 1.54	-3.18^{a} ± 0.48	12.12 ± 1.38	25.37^{c} ± 0.73
Nt267	0	1043	-7.01^{a} ± 0.43	-2.25^{a} ± 0.13	13.56 ± 0.39	28.55^{a} ± 0.21
	1	344	-5.70^{b} ± 0.85	-2.58^{a} ± 0.26	12.85 ± 0.76	27.43^{b} ± 0.40
	2	32	-10.09^{a} ± 2.04	-4.17^{b} ± 0.63	11.64 ± 1.83	24.91^{c} ± 0.97
Q204X	0	1261	-8.90^{a} ± 0.93	-3.69^{a} ± 0.29	13.40^{a} ± 0.84	26.00^{a} ± 0.44
	1	496	-6.30^{b} ± 1.01	-2.30^{b} ± 0.31	11.97^{b}± 0.91	27.92^{b} ± 0.48

$^{a, b}$ means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
0: Homozygous normal, 1: Heterozygous carriers, 2: Homozygous affected
N: Number of animals, SE: Standard Error, Mm: millimetres

Table 4.7 summarises how different genetic variants affected growth traits in the Bonsmara population. Q204X variant significantly affected ($p < 0.05$) BW_{DIR}, WW_{DIR} and FCR resulting in increased BW_{DIR}, WW_{DIR} and reduced FCR. Nt267 genetic variant had no significant effect ($p > 0.05$) on any of the growth traits analysed. WW_{DIR}, ADG and FCR were significantly affected ($p < 0.05$) by both Nt748 and Nt414 variants. Nt748 variant increased the WW_{DIR}, ADG and decreased FCR while Nt414 variant decreased the WW_{DIR}, ADG and increased FCR of the homozygous affected animals compared to the homozygous normal animals. In the case of Nt748 variant, the homozygous affected genotypes seemed to be the superior performing animals while in case of Nt414 variant, the homozygous affected seemed to be

inferior animals.

Table 4.7 Descriptive statistics based on GEBVs for growth traits in the Bonsmara population in this study

Genetic variants	Genotypes	N	Growth traits			
			BW_{DIR} (kg), Mean ± SE	**WW_{DIR} (kg), Mean ± SE**	**ADG (g), Mean ± SE**	**FCR (kg/kg), Mean ± SE**
Clean animals	0000	209	1.18 ± 0.09	15.86 ± 0.42	129.17 ± 4.14	-47.57 ± 1.46
Nt748	0	236	1.25 ± 0.21	15.62^{a} ± 0.88	115.02^{a} ± 8.53	-44.07^{a} ± 2.99
	1	665	1.13 ± 0.15	15.13 ^{a}± 0.63	109.57^{a} ± 6.11	-45.41^{a} ± 2.14
	2	477	1.35 ± 0.11	16.22^{b} ± 0.46	123.30^{b} ± 4.44	-51.74^{b} ± 1.56
Nt414	0	623	1.30 ± 0.11	16.09^{a} ± 0.49	124.26^{a} ± 4.72	-51.18^{a} ± 1.66
	1	535	1.28 ± 0.14	16.21^{a} ± 0.61	120.75^{a} ± 5.90	-50.04^{a} ± 2.07
	2	102	1.14 ± 0.23	14.67^{b} ± 0.99	102.87^{b} ± 9.64	-39.99^{b} ± 3.39
Nt267	0	1043	1.36 ± 0.07	16.23 ± 0.28	124.30 ± 2.72	-50.45 ± 0.95
	1	344	1.34 ± 0.13	16.13 ± 0.54	119.21 ± 5.32	-48.80 ± 1.87
	2	32	1.03 ± 0.31	14.61 ± 1.32	104.37 ±12.80	-41.96 ± 4.49
Q204X	0	1261	1.00^{a} ± 0.14	14.95^{a} ± 0.60	114.22 ± 5.84	-43.74^{a} ± 2.05
	1	496	1.48^{b} ± 0.15	16.37^{b} ± 0.66	117.70 ± 6.37	-50.39^{b} ± 2.24

$^{a, b}$ means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
0: Homozygous normal, 1: Heterozygous carriers, 2: Homozygous affected
N: Number of animals genotyped, SE: Standard Error, KG: Kilogram, G: grams

In Table 4.8 the descriptive statistics based on GEBVs of Nt748, Nt414, Nt267 and Q204X for RTU measurements are shown. Q204X genetic variant had a significant effect ($p < 0.05$) on fat, marbling, and EMA. As a result, heterozygous carrier genotypes had decreased fat and marbling and increased EMA compared to the homozygous normal animals. Both Nt414 and Nt267 variants were significant associated ($p < 0.05$) with both marbling and EMA. Homozygous affected and heterozygous carrier animals of Nt414 had increased marbling and EMA, respectively. Homozygous affected animals for Nt267 variant had increased marbling and EMA. Nt748 variant also had a significant affect ($p < 0.05$) on marbling which resulted in decreased marling content in homozygous affected animals compared to homozygous normal animals.

Table 4.8 Descriptive statistics based on GEBVs for RTU measurements in Bonsmara population in this study

Genetic variants	Genotypes	N	RTU measurements		
			Fat (mm), Mean ± SE	Marbling (mm), Mean ± SE	EMA (cm^2), Mean ±SE
Clean animals	0000	209	-0.34 ± 0.11	0.10 ± 0.06	0.92 ± 0.10
Nt748	0	236	-0.76 ± 0.24	0.25^{a} ± 0.13	1.63 ± 0.21
	1	665	-0.56 ± 0.17	0.14^{a} ± 0.09	1.40 ± 0.15
	2	477	-0.53 ± 0.13	-0.05^{b} ± 0.07	1.55 ± 0.11
Nt414	0	623	-0.68 ± 0.13	-0.07^{a} ± 0.07	1.40^{a} ± 0.12
	1	535	-0.55 ± 0.17	0.16^{b} ± 0.09	1.65^{b} ± 0.15
	2	102	-0.62 ± 0.27	0.25^{b} ± 0.15	1.53^{a} ± 0.24
Nt267	0	1043	-0.56 ± 0.08	-0.05^{a} ± 0.04	1.17^{a} ± 0.07
	1	344	-0.61 ± 0.15	0.07^{ab} ± 0.08	1.42^{b} ± 0.13
	2	32	-0.69 ± 0.36	0.32^{b} ± 0.20	1.98^{b} ± 0.32
Q204X	0	1261	-0.22^{a} ± 0.17	0.32^{a} ± 0.09	1.32^{a} ± 0.15
	1	496	-1.02^{b} ± 0.18	-0.09^{b} ± 0.10	1.74^{b} ± 0.16

[a, b] means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
0: Homozygous normal, 1: Heterozygous carriers, 2: Homozygous affected,
SE: Standard Error, N: Number of animals genotyped, Mm: millimetres, Cm^2: centimetres squared

4.2.2.2 Drakensberger animals

The Levene's Test of Equality of Error Variances that test the assumption of MANOVA and ANOVA that variances of each variable are equal across the groups. Based on the series of Levene's F tests, the homogeneity of variance assumption for ADG, FCR and marbling was violated since the $p < 0.05$. However, the assumption was met for BW_{DIR}, WW_{DIR}, fat, EMA, AFC, ICP, SC and longevity, both at $p > 0.05$. Tables 4.9 indicates the descriptive statistics based on GEBVs for all genetic variants considering traits of economic importance in this study in Drakensberger animals. Nt748 and Nt821 variants had no significant effect ($p > 0.05$) on all the reproduction traits examined in the Drakensberger population. Nt414 variant was the only variant to have a significant effect ($p < 0.05$) on SC in this population, with the heterozygous carrier genotypes displaying a low SC value.

Table 4.9 Descriptive statistics based on GEBVs for reproduction traits in Drakensberger animals

Genetic variants	Genotypes	N	Reproduction traits			
			AFC (months), Mean ± SE	**ICP (days), Mean ± SE**	**SC (mm), Mean ± SE**	**Longevity (months), Mean ± SE**
Clean animals	000	95	-1.92 ± 0.23	-1.89 ± 0.23	12.50 ± 0.74	24.88 ± 0.74
Nt748	0	151	0.52 ± 1.47	-1.85 ± 0.39	10.74 ± 1.30	25.23 ± 0.86
	1	120	-0.94 ± 1.22	-2.01 ± 0.31	11.05 ± 1.08	24.45 ± 0.71
	2	37	-0.84 ± 1.51	-2.31 ± 0.40	11.99 ± 1.34	23.02 ± 0.89
Nt414	0	203	-1.55 ± 1.04	-2.21 ± 0.28	12.69^{a} ± 0.92	24.03 ± 0.61
	1	86	-1.68 ± 1.03	-2.12 ± 0.27	10.04^{b} ± 0.91	24.29 ± 0.60
	2	14	1.97 ± 2.56	-1.84 ± 0.68	11.05^{a} ± 2.27	24.37 ± 1.50
Nt821	0	255	-1.20 ± 0.86	-1.82 ± 0.23	12.08 ± 0.76	24.45 ± 0.50
	1	124	0.36 ± 1.19	-2.29 ± 0.32	10.45 ± 1.06	24.02 ± 0.70

[a, b] means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
0: Homozygous normal, 1: Heterozygous carriers, 2: Homozygous affected
N: Number of animals genotyped, SE: Standard Error, Mm: millimetres

Table 4.10 demonstrate the descriptive statistics based on GEBVs for growth traits in the Drakensberger animals. There was no significant association ($p > 0.05$) between any of the three genetic variants (Nt748, Nt414 and Nt821) and FCR within the Drakensberger animals. BW_{DIR}, WW_{DIR}, and ADG were significantly affected ($p < 0.05$) by Nt821 variant resulting in heterozygous carrier animals showing increased BW_{DIR}, WW_{DIR}, and ADG compared to homozygous normal animals. Heterozygous carrier of Nt821 variant were superior performing animals based on the higher WW_{DIR} and ADG. Nt748 variant had a significant effect ($p < 0.05$) on BW_{DIR} only, with homozygous affected animals displaying higher BW_{DIR} value than the homozygous normal. Nt414 variant also affected ADG significantly ($p < 0.05$), with heterozygous carrier animals showing decreased ADG value compared to both homozygous normal animals.

Table 4.10 Descriptive statistics based on GEBVs for growth traits in Drakensberger animals

Genetic variants	Genotypes	N	Growth traits			
			BW_{DIR} (kg), Mean ± SE	**WW_{DIR} (kg), Mean ± SE**	**ADG (g), Mean ± SE**	**FCR (kg/kg), Mean ± SE**
Clean animals	000	95	-0.06 ± 0.14	0.92 ± 0.55	63.87 ± 5.20	-42.11 ± 2.48
Nt748	0	151	0.11^{a} ± 0.24	9.75 ± 0.94	61.78 ± 9.00	-40.11 ± 4.19
	1	120	0.03^{a} ± 0.19	8.96 ± 0.78	62.78 ± 7.47	-38.62 ± 3.47
	2	37	0.70^{b} ± 0.25	10.74 ± 0.97	67.61 ± 9.28	-43.49 ± 4.31
Nt414	0	203	0.57 ± 0.17	11.01 ± 0.66	74.75^{a} ± 6.36	-45.20 ± 2.96
	1	86	0.37 ± 0.17	9.60 ± 0.66	56.70^{b} ± 6.31	-38.90 ± 2.93
	2	14	-0.10 ± 0.41	8.84 ± 1.64	60.72^{a} ± 15.69	-38.12 ± 7.30
Nt821	0	255	-0.07^{a} ± 0.14	8.29^{a} ± 0.55	57.34^{a} ± 5.26	-38.80 ± 2.45
	1	124	0.63^{b} ± 0.20	11.35^{b} ± 0.76	70.78^{b} ± 7.32	-42.68 ± 3.41

[a, b] means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
0: Homozygous normal, 1: Heterozygous carriers, 2: Homozygous affected
N: Number of animals genotyped, SE: Standard Error, KG: Kilogram, G: grams

The descriptive statistics of the RTU measurements in Drakensberger animals is summarised in Table 4.11. None of the genetic variants in this study had any significant effect ($p > 0.05$) on fat. Also, Nt414 variant showed no significant effect on fat, marbling, or EMA ($p > 0.05$). Nt748 genetic variant only affected marbling significantly ($p < 0.05$) leading to decreased marbling in the homozygous affected animals compared to homozygous normal animals. There was a significant positive relationship ($p < 0.05$) between the Nt821 variant and the EMA in the Drakensberger population which resulted in increased EMA in heterozygous carrier animals compared to homozygous normal animals.

Table 4.11 Descriptive statistics based on GEBVs for RTU measurements in Drakensberger animals

			RTU Measurements		
Genetic variants	**Genotypes**	**N**	**Fat (mm), Mean ± SE**	**Marbling (mm), Mean ± SE**	**EMA (cm^2), Mean ± SE**
Clean animals	000	95	0.07 ± 0.13	0.40 ± 0.08	0.79 ± 0.14
Nt748	0	151	-0.10 ± 0.19	0.43^{a} ± 0.13	1.26 ± 0.24
	1	120	0.03 ± 0.16	0.21^{b} ± 0.11	1.08 ± 0.20
	2	37	0.17 ± 0.20	0.04^{b} ± 0.13	0.82 ± 0.25
Nt414	0	203	0.24 ± 0.14	0.08 ± 0.09	0.95 ± 0.17
	1	86	0.03 ± 0.14	0.28 ± 0.09	0.87 ± 0.17
	2	14	-0.16 ± 0.34	0.32 ± 0.22	1.34 ± 0.43
Nt821	0	255	0.10 ± 0.11	0.30 ± 0.07	0.78^{a} ± 0.14
	1	124	0.06 ± 0.16	0.15 ± 0.10	1.33^{b} ± 0.20

[a, b] means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
0: Homozygous normal, 1: Heterozygous carriers, 2: Homozygous affected
N: Number of animals genotyped, SE: Standard Error, Mm: millimetres (mm), Cm^2: centimetres squared

4.2.3 Additive effect of *MSTN* variants on reproductive, carcass and growth traits of the SA Bonsmara and Drakensberger breeds

4.2.3.1 Bonsmara animals

Some animals displayed more than one *MSTN* variant in their genotype. To analyse the additive effect of more than one variant, the animals with specific combinations were grouped and analysed. Considering the combinations of the four *MSTN* genetic variants, 11 types of *MSTN* variant combinations were found in the Bonsmara population. Among these combinations, animals that were homozygous normal for all the four genetic variants (0000) were compared to those with more than one variant in the *MSTN* gene. The results of the association analysis between the total of 11 combinations and the reproduction traits are summarised in Table 4.12. None of the combined genotypes had any significant effect ($p > 0.05$) on AFC, while combined genotypes GgB 5 and GgB 8 had a significant ($p < 0.05$) effect on ICP which reduced ICP value compared to animals with genotype GgB 0. Only genotype GgB 9 significantly affected ($p < 0.05$) the SC in the SA Bonsmara population, with decreased SC. Six combined genotypes, GgB 1, GgB 2, GgB 5, GgB 6, GgB 8, and GgB 10 had a significant ($p < 0.05$) and negative effect on longevity that reduced the longevity in these animals compared to the animals with genotype GgB 0.

Table 4.12 Mean ± Standard error (SE) of reproductive traits assessed in relation to the combination of the 4 genetic variants in the Bonsmara *MSTN* gene

		Reproductive traits			
Genotype combinations (code)	**Number of animals**	**AFC (months), Mean ± SE**	**ICP (days), Mean ± SE**	**SC (mm), Mean ± SE**	**Longevity (months), Mean ± SE**
0000 (GgB 0)	209	-8.08 ± 0.64	-2.47^{a} ± 0.20	14.55^{a} ± 0.58	29.33^{a}± 0.31
1010 (GgB 1)	145	-8.31 ± 0.77	-2.88^{a} ± 0.24	14.08^{a} ± 0.69	**27.14^{b} ± 0.37**
1100 (GgB 2)	168	-9.03 ± 0.71	-3.16^{a} ± 0.22	13.99^{a} ± 0.64	**27.47^{b} ± 0.35**
1101 (GgB 3)	139	-6.46 ± 0.79	-1.97^{a} ± 0.25	13.42^{a} ± 0.71	29.27^{a} ± 0.38
2010 (GgB 4)	43	-7.68 ± 1.41	-2.00^{a} ± 0.45	16.36^{a} ± 1.27	29.14^{a} ± 0.69
2020 (GgB 5)	30	-13.15 ± 1.69	**-4.21^{b} ± 0.54**	13.11^{a} ± 1.52	**25.49^{b} ± 0.82**
2100 (GgB 6)	59	-6.70 ± 1.21	-3.29^{a} ± 0.38	15.39^{a} ± 1.08	**27.46^{b} ± 0.59**
2101 (GgB 7)	53	-5.88 ± 1.27	-2.08^{a} ± 0.40	13.99^{a} ± 1.14	29.64^{a} ± 0.62
2110 (GgB 8)	60	-10.73 ± 1.20	**-3.93^{b} ± 0.38**	12.62^{a} ± 1.07	**27.12^{b} ± 0.58**
2111 (GgB 9)	42	-3.82 ± 1.43	-1.76^{a} ± 0.45	**8.73^{b} ± 1.28**	27.44^{a} ± 0.69
2200 (GgB 10)	29	-6.30 ± 1.72	-3.19^{a} ± 0.55	14.27^{a} ± 1.54	**24.92^{b} ± 0.84**
2201 (GgB 11)	63	-6.20 ± 1.17	-1.57^{a} ± 0.37	12.25^{a} ± 1.05	28.52^{a} ± 0.57

The combinations are in the following order: Nt748, Nt414, Nt267 and Q204X
Only 11 combinations were studied in the Bonsmara population
$^{a, b}$ means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
Bold mean indicates the group of animals that are significantly different from the control (0000)
Mm: Millimetres, SE: Standard Error, GgB: Genotype group for Bonsmara

Table 4.13 illustrate the analysis of growth traits assessed in relation to the combined genetic variants in the Bonsmara population. None of the 11 combined genotypes showed a significant effect on ADG ($p > 0.05$). Genotype GgB 7 was significantly associated with BW_{DIR}, WW_{DIR} and FCR ($p < 0.05$). An increased BW_{DIR}, and WW_{DIR}, and reduced FCR was observed. Based on the high WW_{DIR} and reduced FCR, these animals were superior performing animals. Other combined genotypes that were significantly

associated with FCR were GgB 3, GgB 6 and GgB 9 ($p < 0.05$) and the decreased FCR in these animals was favourable.

Table 4.13 Mean ± Standard error (SE) of growth traits assessed in relation to the combination of four genetic variants in the Bonsmara *MSTN* gene

Genotype combinations (code)	Number of animals	Growth traits			
		BW_{DIR} (kg), Mean ± SE	**WW_{DIR} (kg), Mean ± SE**	**ADG (g), Mean ± SE**	**FCR (kg/kg), Mean ± SE**
0000 (GgB 0)	209	1.18^{a} ± 0.09	15.86^{a} ± 0.42	129.17 ± 4.11	-47.57^{a} ± 1.43
1010 (GgB 1)	145	1.08^{a} ± 0.12	15.63^{a} ± 0.50	122.68 ± 4.93	-47.30^{a} ± 1.71
1100 (GgB 2)	168	1.02^{a} ± 0.11	15.35^{a} ± 0.47	118.44 ± 4.58	-47.41^{a} ± 1.59
1101 (GgB 3)	139	1.53^{a} ± 0.12	17.54^{a} ± 0.51	131.11 ± 5.04	**-57.25^{b} ± 1.75**
2010 (GgB 4)	43	1.38^{a} ± 0.21	17.24^{a} ± 0.93	144.32 ± 9.06	-56.91^{a} ± 3.15
2020 (GgB 5)	30	0.95^{a} ± 0.25	14.90^{a} ± 1.11	118.26 ± 10.84	-47.41^{a} ± 3.77
2100 (GgB 6)	59	1.42^{a} ± 0.18	17.05^{a} ± 0.79	139.93 ± 7.73	**-56.36^{b} ± 2.69**
2101 (GgB 7)	53	**1.88^{b} ± 0.19**	**18.59^{b} ± 0.83**	145.47 ± 8.16	**-60.91^{b} ± 2.83**
2110 (GgB 8)	60	0.95^{a} ± 0.18	15.83^{a} ± 0.78	125.31 ± 7.67	-51.50^{a} ± 2.66
2111 (GgB 9)	42	1.83^{a} ± 0.21	16.65^{a} ± 0.94	110.94 ± 9.16	**-57.40^{b} ± 3.18**
2200 (GgB 10)	29	1.55^{a} ± 0.26	16.63^{a} ± 1.13	125.92 ± 11.03	-49.12^{a} ± 3.83
2201 (GgB 11)	63	1.39^{a} ± 0.18	15.70^{a} ± 0.76	114.79 ± 7.48	-48.83^{a} ± 2.60

The combinations are in the following order: Nt748, Nt414, Nt267 and Q204X
Only 11 combinations were studied in the Bonsmara population
$^{a, b}$ means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
Bold mean indicates the group of animals that are significantly different from the control (0000)
KG: Kilogram, G: gram, SE: standard error, GgB: Genotype groups for Bonsmara

The results of the association analysis between RTU measurements and combined genotypes in the Bonsmara *MSTN* gene are summarised in Table 4.14. Genotype GgB 3 had a significant effect on both marbling and EMA ($p < 0.05$) which caused marbling content to decrease and EMA content to increase. GgB 11 affected both fat and marbling significantly ($p < 0.05$) and both fat content and marbling content

decreased compared to the GgB 0 effect. Genotypes GgB 7, GgB 9 and GgB 10 had a significant effect ($p < 0.05$) EMA, marbling, and fat, with increased EMA, decreased marbling and increased fat, respectively.

Table 4.14 Mean ± Standard error (SE) of RTU measurements assessed in relation to the combination of the four genetic variants in the Bonsmara *MSTN* gene

		RTU measurements		
Genotype combinations (codes)	**Number of animals**	**Fat (mm), Mean ± SE**	**Marbling (mm), Mean ± SE**	**EMA (cm^2), Mean ± SE**
0000 (GgB 0)	209	-0.34^{a} ± 0.12	0.10^{a} ± 0.06	0.92^{a} ± 0.10
1010 (GgB 1)	145	-0.17^{a} ± 0.14	0.17^{a} ± 0.08	1.10^{a} ± 0.12
1100 (GgB 2)	168	-0.11^{a} ± 0.13	0.22^{a} ± 0.07	1.02^{a} ± 0.11
1101 (GgB 3)	139	-0.57^{a} ± 0.14	**-0.19^{b} ± 0.08**	**1.37^{b} ± 0.12**
2010 (GgB 4)	43	0.12^{a} ± 0.25	-0.15^{a} ± 0.14	1.25^{a} ± 0.22
2020 (GgB 5)	30	-0.26^{a} ± 0.30	0.17^{a} ± 0.17	1.68^{a} ± 0.26
2100 (GgB 6)	59	-0.11^{a} ± 0.22	-0.06^{a} ± 0.12	1.12^{a} ± 0.19
2101 (GgB 7)	53	-0.91^{a} ± 0.23	-0.11^{a} ± 0.12	**1.95^{b} ± 0.20**
2110 (GgB 8)	60	-0.04^{a} ± 0.22	-0.24^{a} ± 0.12	1.18^{a} ± 0.19
2111 (GgB 9)	42	-1.12^{a} ± 0.26	**-0.44^{b} ± 0.14**	1.30^{a} ± 0.22
2200 (GgB 10)	29	**0.51^{b} ± 0.31**	0.19^{a} ± 0.17	1.00^{a} ± 0.27
2201 (GgB 11)	63	**-1.17^{c} ± 0.21**	**-0.32^{b} ± 0.11**	1.38^{a} ± 1.18

The combinations are in the following order: Nt748, Nt414, Nt267 and Q204X
Only 11 combinations were studied in the Bonsmara population
$^{a, b}$ means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
Bold mean indicates the group of animals that are significantly different from the control (0000)
Mm: Millimetre, Cm^2: Centimetre, SE: Standard Error, GgB: Genotype groups for Bonsmara

4.2.3.2 Drakensberger animals

In the combination's analysis, the descriptive statistics of reproductive traits assessed in relation to the combination of the three genetic variants in the *MSTN* gene of Drakensberger is shown in Table 4.15. None of the combined genotypes had a significant effect on AFC, ICP or longevity ($p > 0.05$). SC was the

only trait that was significantly affected by the genotype GgD 03 in the Drakensberger population ($p < 0.05$), and these animals displayed significant lower SC than all other genotypes.

Table 4.15 Mean ± standard error (SE) of reproductive traits assessed in relation to the combination of the 3 genetic variants in the Drakensberger *MSTN* gene

		Reproduction traits			
Genotype combinations (codes)	**Number of animals**	**AFC (months), Mean ± SE**	**ICP (days), Mean ± SE**	**SC (mm), Mean ± SE**	**Longevity (months), Mean ± SE**
000 (GgD 00)	95	-1.92 ± 0.87	-1.89 ± 0.23	12.51^{a} ± 0.74	24.88 ± 0.49
101 (GgD 01)	10	-3.44 ± 2.67	-2.83 ± 0.71	12.05^{a} ± 2.29	22.23 ± 1.50
110 (GgD 02)	46	-2.72 ± 1.25	-1.74 ± 0.33	11.74^{a} ± 1.07	25.20 ± 0.70
111 (GgD 03)	19	-2.88 ± 1.94	-2.71 ± 0.51	**6.42^{b} ± 1.66**	23.42 ± 1.09
210 (GgD 04)	21	-2.15 ± 1.84	-2.00 ± 0.49	11.55^{a} ± 1.58	23.07 ± 1.03
220 (GgD 05)	14	0.77 ± 2.26	-1.86 ± 0.60	12.60^{a} ± 1.94	23.37 ± 1.26

The combinations are in the following order: Nt748, Nt414, and Nt821
Only five combinations were studied in the Drakensberger population
[a, b] means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
Bold mean indicates the group of animals that are significantly different from the control (000)
0: Homozygous normal, 1: Heterozygous carriers, 2: Homozygous affected
SE: Standard Error, Mm: millimetre, GgD: Genotype groups for Drakensberger

Table 4.16 illustrate the descriptive statistics of growth traits assessed in relation to the combination of the three genetic variants in the Drakensberger *MSTN* gene. In the analysis of the combinations, none of the combined genotypes had a significant effect on any of the traits of interest in this study ($p > 0.05$). However, these results showed a trend were animals with genotype GgD 01 seemed to be superior performing due to increased WW_{DIR}, high ADG and decreased FCR.

Table 4.16 Mean ± standard error (SE) of growth traits assessed in relation to the combination of the three genetic variants in the Drakensberger *MSTN* gene

Genotype combinations (codes)	Number of animals	Growth traits			
		BW_{DIR} (kg), Mean ± SE	**WW_{DIR} (kg), Mean ± SE**	**ADG (g), Mean ± SE**	**FCR (kg/kg), Mean ± SE**
000 (GgD 00)	95	-0.06 ± 0.14	9.02 ± 0.54	63.87 ± 4.99	-42.10 ± 2.34
101 (GgD 01)	**10**	**0.65 ±0.43**	**11.35 ± 1.65**	**82.21 ± 15.38**	**-49.22 ± 7.21**
110 (GgD 02)	46	-0.04 ± 0.20	7.96 ± 0.77	53.07 ± 7.17	-36.42 ± 3.36
111 (GgD 03)	19	-0.11 ± 0.31	8.50 ± 1.20	51.69 ± 11.15	-33.86 ± 5.23
210 (GgD 04)	21	0.55 ± 0.29	8.94 ± 1.14	53.43 ± 10.61	-40.64 ± 4.97
220 (GgD 05)	14	-0.02 ± 0.36	8.24 ± 1.40	57.55 ± 12.99	-38.93 ± 6.09

The combinations are in the following order: Nt748, Nt414, and Nt821
Only five combinations were studied in the Drakensberger population
[a, b] means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
Bold mean indicates the group of animals that are significantly different from the control (000)
0: Homozygous normal, 1: Heterozygous carriers, 2: Homozygous affected
SE: Standard Error, KG: Kilogram, G: gram, GgD: Genotype groups for Drakensberger

Descriptive statistics of the RTU measurements assessed in relation to the combination of the three genetic variants in the Drakensberger *MSTN* gene are summarised in Table 4.17. The result found no significant association between any of the combined genotypes (GgD 01, GgD 02, GgD 03, GgD 04 and GgD 05) and any of the traits (fat, marbling, and EMA) in this study ($p > 0.05$).

Table 4.17 Mean ± standard error (SE) of RTU measurements assessed in relation to the combination of the 3 genetic variants in the Drakensberger *MSTN* gene

Genotype combinations (codes)	Number of animals	RTU measurements		
		Fat (mm), Mean ± SE	**Marbling (mm), Mean ± SE**	**EMA (cm^2), Mean ± SE**
000 (GgD 00)	95	0.07 ± 0.11	0.40 ± 0.07	0.79 ± 0.13
101 (GgD 01)	10	0.13 ± 0.33	0.04 ± 0.21	0.90 ± 0.41
110 (GgD 02)	46	0.53 ± 0.15	0.25 ± 0.10	0.76 ± 0.19
111 (GgD 03)	19	0.09 ± 0.24	0.37 ± 0.15	0.89 ± 0.30
210 (GgD 04)	21	-0.01 ± 0.23	0.17 ± 0.14	0.29 ± 0.28
220 (GgD 05)	14	-0.05 ± 0.28	0.20 ± 0.18	0.83 ± 0.34

The combinations are in the following order: Nt748, Nt414, and Nt821

Only five combinations were studied in the Drakensberger population
[a, b] means in the same column bearing the different superscript differ significantly at $p < 0.05$ per variant
Bold mean indicates the group of animals that are significantly different from the control (000)
0: Homozygous normal, 1: Heterozygous carriers, 2: Homozygous affected
SE: Standard Error, Mm: millimetre, Cm^2: centimetres squared, GgD: Genotype groups for Drakensberger

Chapter 5 Discussion

5.1 Introduction

The ultimate goal of most modern beef cattle production systems is to increase production efficiency to remain competitive, economically viable, and meet the growing demand for meat. The availability of genomic data and reliable phenotypic recording of performance traits are both required for genetic improvement in livestock species (Visser *et al.*, 2020). The current genomic tools, performance recording systems, and advanced genetic evaluation used to determine GEBVs or EBVs contributed to the genetic improvement of the economically important traits in beef industry. Association analysis method is the most frequently used method to link phenotypes and genotypes to candidate genes such as the *MSTN* gene. This study was requested by the SA Breeders' societies after they observed double muscling syndrome in their herds.

5.2 Observed *MSTN* variants and their prevalence

The results from the pilot study indicated the presence of Nt821, F94L and Q204X variants in the Bonsmara Beefmaster, Brangus, Drakensberger and Limousin populations. The genotypic frequencies of *MSTN* variants across 355 animals ranged from 0.00% (Nt821 variant) in Bonsmara to 100% (F94L variant) in Limousin among the homozygous affected animals. Among the heterozygous carrier animals, genotypic frequencies ranged from 0.00% (F94L) in Limousin to 52% (Nt821) in Beefmaster cattle. The most prevalent variant was the F94L variant with 100% genotypic frequency of homozygous affected animals in the Limousin breed and nearly absent in other South African breeds. In addition, the frequency of the desirable recessive (q) allele in this study was the highest (100%) in the Limousin populations. This was consistent with several studies (Dunner *et al.*, 2003; Lee *et al.*, 2015; Bennett *et al.*, 2019; Anwar *et al.*, 2020; Konovalova *et al.*, 2021) where F94L was reported as the desirable allele at a 90-100% frequency in Limousin cattle. In phase two of the study, four and three of the known variants were observed in the SA Bonsmara and Drakensberger breeds, respectively. The most prevalent variant was Nt748, followed by Nt414 in homozygous affected animals in both SA Bonsmara and Drakensberger populations. Other researchers (Groblet *et al.*, 1998; Smith *et al.*, 2000; Dunner *et al.*, 2003; Marchitelli, 2003; Allais *et al.*, 2010; Konovalova *et al.*, 2021) reported that the Nt821*(del 11)* variant was the most common variant in double-muscled animals across different cattle breeds but mostly fixed in Belgian Blue cattle because of long-term selection for increased muscle mass (Konovalova *et al.*, 2021). However, in the current study, the Nt821*(del 11)* was the least common variant found in the Drakensberger breed.

5.3 Association analysis

5.3.1 Reproductive traits

Reproduction traits are of economic importance due to the fact that cows that calve early in life and have regular calving intervals produce more calves in their lifetime, resulting in an increase in the replacement rate of females and overall production (Patterson *et al.*, 2016). Reproductive difficulties in double muscled cattle has long been known and reported in the literature (Arthur, 1995; De Smet, 2004). The majority of research on the reproduction of double-muscled female animals focuses on decreased fertility, increased dystocia, increased calving difficulties, and reduced milk production (Arthur, 1995; Fiems, 2012). However, the most prominent difference in terms of reproduction is the well-documented increased frequency of calving difficulties (dystocia) due to a feto-maternal morphological imbalance at calving. The issue is thought to be caused by a combination of two factors: excessive muscular development of double-muscled calves and underdeveloped maternal reproductive tract of the double-muscled dam (Arthur, 1995; Kambadur *et al.*, 2004). As a result, frequent caesarean births are required to circumvent increased incidence of dystocia (Kambadur *et al.*, 2004; Allais *et al.*, 2010; Tuska *et al.*, 2021). Additionally, reduced fertility in double-muscled females is mainly reflected by a later age at first calving, longer intervals between calving and first oestrus, and a large number of services per pregnancy (De Smet, 2004).

The reproduction traits in this study include age at first calving (AFC), inter-calving period (ICP), scrotal circumference (SC), and longevity in SA Bonsmara and Drakensberger. The variants that significantly affected ($p < 0.05$) AFC in SA Bonsmara cattle were Nt748, Nt267 and Q204X while none of the genetic variants of interest in this study had an effect on AFC in the Drakensberger cattle ($p > 0.05$). In SA Bonsmara, AFC of homozygous affected animals for Nt748 variant (homozygous affected vs homozygous normal) and Nt267 variant (homozygous affected vs heterozygous carrier) was shorter in this study. These results are similar to those reported in the Asturiana de los Valles cattle breed with Nt821 variant where the difference between double muscled cattle (931 days) and normal (969 days) for AFC was 38 days (Cafion *et al.*, 2002). In contrast, it was reported that double muscled animals carrying Nt821 variant in Belgian Blue breed had longer AFC (De Smet, 2004). This study also found that heterozygous carriers of Q204X variant had later AFC compared to homozygous normal animals. No previously published results are directly comparable with the results of this study. Earlier AFC has been reported to be a consequence of the correlated incidence of caesarean sections (Cafion *et al.*, 2002), while later AFC was a result of reduced fertility (De Smet, 2004). The overall number of calves produced in a lifetime will drop in females who calve later in life, resulting in a decrease in productivity (Ahlberg *et al.*, 2016).

Maintaining a short inter-calving interval (ICP) in beef cows is a key goal for maximizing calf production and reducing calf mortality (Fiems and Ampe, 2015). None of the genetic variants of interest

had a significant impact on ICP in Drakensberger cattle ($p > 0.05$). Within the SA Bonsmara population, homozygous affected for Nt414 variant animals had a longer ICP compared to heterozygous carrier animals. ICP results of homozygous affected animals of the SA Bonsmara breed in our study was relatively similar to what has been reported in Belgian Blue (Hanzen *et al.*, 1994; De Smet, 2004; Fiems *et al.*, 2006) and in Asturiana de los Valles cattle (Cafion *et al.*, 2002) where homozygous affected animals had longer ICP compared to the homozygous normal animals. Also, heterozygous carriers of the Q204X variant had longer ICP compared to homozygous normal animals. However, direct comparison of cattle that are heterozygous carriers of any *MSTN* genetic variants and homozygous normal cattle are limited in literature. Reasons for longer ICP might be due to the lower conception rate, lower pregnancy rate, lower calving rate (Ménissier, 1982; Fiems and Ampe, 2015), caesarean at parturition and the development of perimetrium adhesions (Fiems *et al.*, 2006) observed in homozygous affected animals. Observing the effect of the Nt267 variant on ICP in SA Bonsmara cattle, the homozygous affected animals had a shorter ICP than homozygous normal and heterozygous carrier cattle. This was the opposite of what has been reported in literature, leading to Nt267 variant having a positive and favourable effect on ICP. The herd's productivity may increase as a result of the shorter ICP since more calves may be produced per cow over the course of her lifetime (Boligon *et al.*, 2016).

Scrotal circumference (SC) is known to be a good indication of fertility in bulls. SC of the Bonsmara cattle was only affected by the Q204X variant and the Nt414 variant only affected the SC of the Drakensberger cattle. In both populations, the heterozygous carrier cattle were found to have smaller SC compared to the homozygous normal cattle. In literature, there is limited evidence to support our findings but most researchers (Michaux and Hanset, 1981; Bellinge *et al.*, 2005; Hoflack *et al.*, 2006; Hoflack *et al.*, 2008; Garcia-Paloma, 2015) reported double muscling condition in Belgian Blue to be associated with a smaller SC, poor semen quality and reduced semen production. This was suggested to be due to testicular hypoplasia or degeneration in the double muscled Belgian Blue breed (Hoflack *et al.*, 2008; Yimer *et al.*, 2011; Foster, 2016; Kouamo and Nyonga, 2022). However, in this study, the homozygous affected Drakensberger animals for Nt414 variant had larger SC when compared to the heterozygous carrier animals. Larger SC results in increased semen production and semen quality (Hoflack *et al.*, 2008). In order to make long-term selection for heavier bulls, it is crucial to pay closer attention to any potential negative or positive associations between SC and body weight (van Marle-Köster *et al.*, 2000).

Longevity is one of the most economically importance traits in beef production. Longevity of cattle is difficult to measure because it is expressed later in the life of a cow and because of its correlation with many other important traits (Imbayarwo-Chikosi *et al.*, 2015). The longevity of the SA Bonsmara cattle in this study was significantly affected by all variants of interest (Nt748, Nt414, Nt267 and Q204X) while none of the variants of interest (Nt748, Nt414 and Nt821) affected longevity in the Drakensberger cattle.

In SA Bonsmara breed, Nt748 variant affected longevity of the heterozygous carrier unfavourably with decreased longevity value compared to both homozygous groups while Q204X variant affected longevity of heterozygous carriers favourably with increased longevity compared to homozygous normal animals. Both Nt414 and Nt267 variants unfavourably affected longevity showing a decrease in longevity values. None of the aforementioned effects of *MSTN* variants reported in the Bonsmara cattle was reported previously in other cattle breeds. In the case of Nt267, the number of animals genotyped (n=32) might have contributed to these results. Given the significant effect of the Nt414 and Nt267 variants on longevity of homozygous affected cattle, these two variants were unfavourable and will adversely affect the trait. It is clear that the effect on reproduction depends on the variant, as well as the population in which it occurs.

5.3.2 Growth traits

The effect of double muscling on growth traits has been explored and well documented in literature (Arthur, 1995; De Smet, 2004; Wiener *et al.*, 2009; Flynn and Flynn, 2015). The results in this study showed that the direct birth weight (BW_{DIR}) of the Bonsmara cattle was only affected by Q204X while Nt748 and Nt821 variants affected the BW_{DIR} of the Drakensberger cattle. Q204X and Nt821 variants increased BW_{DIR} in heterozygous carrier animals compared to homozygous normal animals in Bonsmara and Drakensberger breed, respectively. Similarly, increased birth weight was also observed in heterozygous carriers of C313Y variant in Piedmontese (Casas *et al.*, 1999) and Piedmontese cross calves (Short *et al.*, 2002), Nt821 variant in Belgian Blue calves (Casas *et al.*, 1998), Belgian Blue × British and Charolais calves (Casas *et al.*, 2004), and Q204X variant in Charolais calves (Allais *et al.*, 2010). Among the Drakensberger cattle, calves that were homozygous affected by Nt748 variant were heavier at birth compared to the homozygous normal calves. These results follow the general trend that has been previously reported by the majority of researchers (Hanset, 1991; Arthur, 1995; Bellinge *et al.*, 2005; Wang *et al.*, 2015) in double-muscled animals. Additionally, higher birth weight were also observed in homozygous affected animals for Nt821 variant in Belgian Blue calves (Ménissier, 1982; Arthur, 1995; Fiems *et al.*, 2001; De Smet, 2004), Belgian Blue × British calves (Casas *et al.*, 2004), German Gelbvieh calves (Dierks *et al.*, 2015; Konovalova *et al.*, 2021), Asturiana de los Valles calves (Cafion *et al.*, 2002), South Devon calves (Wiener *et al.*, 2009) as well as for F94L variant in Limousin cattle (Esmailizadeh *et al.*, 2008) compared to homozygous normal animals. Konovalova *et al.* (2021) found no significant effect of F94L variant on birth weight in Limousin backcross calves in Australian and New Zeeland populations. High direct birth weight have an impact on calf survival and is correlated to increased culling, decreased fertility as well as dystocia (Lochner, 2018). In the current study, the Q204X variant in SA Bonsmara animals and, the Nt748 and Nt821 variants in Drakensberger animals had unfavourable effect on BW_{DIR} and will lead to caesarean sections due to calving difficulties or a high frequency of dystocia.

The main source of farm income for commercial farmers is the weaning weight of the calves given an adequate level of fertility (Miller and Wilton, 1999), which is determined by both the calf's genetic potential (direct influence) and the dam's effect (maternal effect) at the genetic level (Cortés-Lacruz *et al.*, 2017; Lochner, 2018). In this study, WW_{DIR} of the Bonsmara cattle was significantly affected by Nt748, Nt414 and Q204X variants while in the Drakensberger cattle, only Nt821 variant affected the WW_{DIR}. The Q204X-heterozygous carriers in SA Bonsmara cattle and Nt821-heterozygous carriers in Drakensberger cattle were heavier at weaning compared to the homozygous normal cattle. Similar results were reported in heterozygous carriers of C313Y variant in Piedmontese cattle (9.1 vs. -8.4 kg) (Casas *et al.*, 1999) and heterozygous carriers of Nt821 variant in Charolaise and Belgian Blue × British animals (Casas *et al.*, 2004) when compared to homozygous normal cattle. However, other studies (Arthur, 1995; Short *et al.*, 2002) found no significant difference between the weaning weights of heterozygous carrier animals and homozygous normal animals. In this study, calves that were also heavier at weaning were those that were homozygous affected for Nt748 variant in the SA Bonsmara population. Similar observations were reported in Belgian Blue cattle (Arthur, 1995) and Asturiana de los Valles (Cafion *et al.*, 2002) where homozygous affected animals for Nt821 variant were compared to homozygous normal animals. In contrast, Nt414-homozygous affected Bonsmara cattle were lighter at weaning compared to homozygous normal cattle. These results were not consistent with results reported previously in literature. In addition, some other studies found no significant difference between the weaning weight of homozygous affected and homozygous normal animals in Piedmontese (Casas *et al.*, 1999) and in Belgian Blue × British Breed (Casas *et al.*, 2004). This study revealed that the Nt748, Q204X and Nt821 variants had favourable effect while Nt414 variant had unfavourable effect associated with weaning weight within the SA Bonsmara and Drakensberger population.

The confounding effects between *MSTN* variants and breed effects are the most likely cause of these contradictory results and weights that are expressed early in life are influenced by maternal effects that may change performance (Casas *et al.*, 2004). Given the effect of the *MSTN* variants on weaning weight of heterozygous carrier cattle, it is advisable to use production systems that uses mature cows to produce heterozygous carrier calves to benefit from heavier weaning weight compared to homozygous normal calves, while avoiding calving difficulties reported in homozygous affected cattle (Casas *et al.*, 2004). In this study, Q204X and Nt821 variants increased BW_{DIR} while increasing WW_{DIR} of heterozygous carriers in both the SA Bonsmara and Drakensberger breed. In the SA Bonsmara breed, Nt748 and Nt414 increased and decreased WW_{DIR} of homozygous affected animals without affecting the BW_{DIR}, respectively.

Average daily gain (ADG) in the beef cattle industry is considered as a trait that influences production efficiency (Day and Nogueira, 2013). Nt748 and Nt414 variants influenced the ADG of the Bonsmara cattle while the ADG among the Drakensberger cattle was influenced by Nt414 and Nt821

variants. The Nt748 variant increased ADG while Nt414 variant decreased ADG in homozygous affected animals compared to the homozygous normal animals within the Bonsmara breed population. The effect of the Nt748 variant in this study is consistent with the effect of F94L variant in Limousin cattle (Hales *et al.*, 2020) and Nt821 in Asturiana cattle (Cafion *et al.*, 2002) where homozygous affected animals had a greater ADG compared to homozygous normal animals. In contrast, decreased ADG observed in this study in homozygous affected animals for Nt414 variant in Bonsmara cattle was consistent with the observation made in homozygous affected animals for Nt821 variant in Belgian Blue cattle (Meyermans *et al.*, 2022). However, homozygous affected animals had similar ADG to homozygous normal animals in Belgian Blue × British breed (Casas *et al.*, 2004). Within the Drakensberger breed, Nt414 decreased the ADG while Nt821 variant increased ADG in heterozygous carrier animals. Nt414-heterozygous carrier animals had lower ADG compared to both homozygous groups while Nt821-heterozygous carrier animals had increased ADG compared to homozygous normal animals. In the literature, there is no research with similar results obtained in this study. However, Casas *et al.* (2004) found that Nt821-heterozygous carriers and homozygous normal animals had similar ADG levels in Charolais and Belgian Blue × British animals. The discrepancy between the effects of genetic variants on ADG in difference breeds across studies might be due to the feeds that animals were being fed during studies.

Feed conversion ratio (FCR) is an efficient way to maximize production and efficiency in beef production (Besson *et at.*, 2016). The results of this study revealed that none of the genetic variants affected the FCR in Drakensberger cattle. Among the SA Bonsmara cattle, FCR was significantly affected by Nt748, Nt414 and Q204X variants. Nt748 variant affected FCR of the homozygous affected animals in this study favourably, which was similar to the effect of Nt821 variant in homozygous affected animals compared to homozygous normal animals in Belgian Blue cattle (Hanset, 1991; Casas *et al.*, 1998; Cundiff *et al.*, 1998; Grobet *et al.*, 1998; De Smet, 2004; Boukha, 2008). Contrary to this, Nt414 variant showed an unfavourable effect on FCR with increased FCR values in homozygous affected animals compared to homozygous normal animals. In Q204X-heterozygous carriers, FCR was decreased compared to the homozygous normal animals in this study. Similar effects as of those of Nt414 and Q204X variants in this study was not previously reported in research in any double-muscled cattle breeds. The reduced FCR in double-muscled animals are most likely owing to a shift in the composition of body weight gains toward more protein and less fat deposition, rather than changes in feed digestibility or maintenance requirements (De Smet, 2004). Nt748-homozygous affected and Q204X-heterozygous carrier animals in this study performed better than homozygous normal animals in terms of WW_{DIR}, ADG and FCR while Nt414-homozygous animals were inferior for the same traits.

5.3.3 Carcass traits based on RTU measurements

Beef cattle breeders have an interest in carcass traits due to the effect on the yield and quality of the finished product. Additionally, fat, meat tenderness and meat yield are significant in beef production as they determine eating quality as well as the price of meat (Seroba *et al.*, 2011; Bureš and Bartoň, 2012). Marbling score, fat thickness, dressing percentage, and hot carcass weight are a few traits that are used to measure carcass quality and these traits can be categorised as cutability and quality traits (Lochner, 2018). Muscling and leanness are quality traits, while marbling is a representation of cutability traits (Lochner, 2018). The beef cattle farmer faces a number of hurdles when it comes to genetic improvement of carcass traits, since these traits are difficult and expensive to measure (Hocquette *et al.*, 2007).

A number of studies has reported the influence of different *MSTN* variants on fat content in various cattle breeds (Esmailizadeh *et al.*, 2008; Aiello *et al.*, 2018; Konovalova *et al.*, 2021; Ceccobelli *et al.*, 2022). Despite this, none of the *MSTN* variants had an effect on the fat content of the Drakensberger cattle in this study. The Q204X variant in this study was the only variant to have a significant effect on fat content of the Bonsmara cattle where decreased fat content was observed in the heterozygous carrier cattle compared to homozygous normal. O'Rourke *et al.* (2009) found similar results in heterozygous carriers of Nt821 in Angus × Hereford cattle. This could be due to the fact that Q204X and Nt821 variants are categorised as disruptive variants. In this study, the fat of the homozygous affected animals was not affected by any variants in either SA Bonsmara or Drakensberger cattle, which is inconsistent with what has been previously reported in literature. Several researchers (Arthur, 1995; Raes *et al.*, 2001; Wiener *et al.*, 2002; De Smet, 2004; Webb and Casey, 2010; Fiems, 2012; Renna *et al.*, 2019) reported double-muscled cattle with low fat content compared to normal cattle. For example, Nt821 variant in South Devon cattle reduced fat content in homozygous affected animals (4.04%) compared to the homozygous normal (6.81%) and heterozygous carrier animals (5.60%) (Wiener *et al.*, 2002). It is possible that Nt748, Nt414 and Nt267 variants did not have any effect on the fat content in this study because they are known to be silent variants without any significant effect.

The results in the Bonsmara population showed that all genetic variants of interest (Nt748, Nt414, Nt267 and Q204X) significantly affected marbling while in the Drakensberger population, marbling was only affected by Nt748 variant. In both SA Bonsmara and Drakensberger populations, animals that were found to be homozygous affected for Nt748 variant had a lower marbling content than the homozygous normal cattle. This is an unfavourable effect since a decrease in marbling results in decreased flavour, juiciness and tenderness which affect consumers' preference (Mateescu *et al.*, 2015; Mwangi *et al.*, 2019). The results of the current study were consistent with what has been previously reported, namely that double muscled animals tend to produce tough meat due to reduced marbling compared to homozygous normal animals (Culley, 1807; MacKellar, 1960; Clinquart *et al.*, 1998). Although some studies reported marbling

to be significantly affected by the *MSTN* genetic variants others have found no significant effect of double muscling on carcass marbling (Han *et al.*, 2012; Konovalova *et al.*, 2021).

Heterozygous carriers of Q204X variant also had lower marbling content when compared to homozygous normal cattle. Other researchers (Casas *et al.*, 1998; Wiener *et al.*, 2002; O'Rourke *et al.*, 2009) also found the heterozygote animals in their studies to have decreased marbling compared to homozygous normal cattle. For example, O'Rourke *et al.* (2009) found heterozygous carriers of the Nt821 variant in Angus × Hereford cross animals to be 25% lower in marbling content when compared to homozygous normal, while in Angus and Charolais herds there were no significant difference between heterozygotes and homozygous wildtype.

Nt414 and Nt267 variants in the Bonsmara population affected marbling favourably with higher marbling content in homozygous affected cattle than in homozygous normal cattle. Increased marbling result in increased juiciness, flavour, and tenderness. This agrees with several reports indicating that the meat from double-muscled cattle is more tender than in homozygous normal animals (Arthur, 1995; Wheeler *et al.*, 2001; Boukha *et al.*, 2011; Fiems, 2012). Increased meat tenderness in double-muscled animals is reported to be caused by lower collagen content and a lower proportion of stable non-reducible crosslinks (O'Rourke *et al.*, 2009; Fiems, 2012). High carcass marbling content is important since it is believed to play a significant role in consumer satisfaction, enhanced profitability as well as determining the palatability of beef (Mateescu *et al.*, 2015; Gotoh *et al.*, 2018). There is no literature available that shows the correlation between backfat and marbling in other double-muscled cattle breeds. However, this study indicates that while the fat content of the Bonsmara cattle was not affected by Nt748, Nt414 or Nt267 variants, the same variants affected the marbling of the same animals.

Carcass yield and carcass weight can be determined using eye muscle area (EMA) (Medeiros de Oliveira Silva *et al.*, 2017). The EMA in this study was significantly affected by Nt414, Nt267 and Q204X in SA Bonsmara cattle while EMA in Drakensberger cattle was only affected by Nt821 variant. Greater EMA was observed in heterozygous carriers of Q204X, Nt414 variants in SA Bonsmara and Nt821 variant in Drakensberger cattle. Similar results were obtained in heterozygous carriers of Nt821 variant in Angus-sired cattle (Gill *et al.*, 2009), Belgian Blue cross (Casas *et al.*, 1998) and Angus × Hereford calves and cows (Casas *et al.*, 1998). The EMA within the SA Bonsmara cattle population was greater in homozygous affected animals compared to homozygous normal animals. This observation was consistent with the effect of F94L variant in the Limousin breed (Sellick *et al.*, 2007; Esmailizadeh *et al.*, 2008) and Nt821 variant in Angus × Hereford cross (O'Rourke *et al.*, 2009). In addition, Han *et al.* (2012) found no significant difference between the EMA of homozygous affected, heterozygous carrier and homozygous normal cattle. EMA and slaughter weight are correlated as an increased muscle yield will be associated with an increase in the EMA (Miar *et al.*, 2014). The results of the Q204X variant in heterozygous carrier in SA Bonsmara

in the present study confirms the reports in literature that heterozygous carriers of different *MSTN* variants have low fat content, more muscling with increased EMA compared to homozygous normal animals (Casas *et al.*, 1998; Sellick *et al.*, 2007; Esmailizadeh *et al.*, 2008; Gill *et al.*, 2009; O'Rourke *et al.*, 2009) which result in higher carcass yield. The reason for this outcome might be that Q204X variant is categorised as a disruptive variant.

In conclusion, Nt267 variant in this study can be used as a possible genetic marker of productivity traits due to its favourable effect on AFC, ICP, marbling and EMA of the homozygous affected animals while it does not have any effect on other traits such as BW_{DIR}; therefore, the dystocia frequency should not increase.

5.4 Additive effect of *MTSN* variants on reproductive, carcass and growth traits of the SA Bonsmara and Drakensberger breeds

This is the first study to report the impact of combined *MSTN* variants on reproduction, growth, and carcass traits in the SA Bonsmara and Drakensberger breeds. This study identified eleven combinations in Bonsmara cattle and five in Drakensberger cattle. The genotype 0000 (GgB 0) in Bonsmara breed and 000 (GgD 00) in Drakensberger breed were compared to the rest of the combinations.

The results of the genotype combinations in this study showed that having more than one variant in the *MSTN* gene could have a favourable or unfavourable effect on some traits. In the SA Bonsmara population, both genotype 2020 (GgB 5) and 2110 (GgB 8) had a favourable influence on ICP but unfavourable influence on longevity. Also, genotype 2100 (GgB 6) affected longevity unfavourably but affected FCR favourably. Genotype 2111 (GgB 9) had a negative effect on SC and marbling while FCR was positively affected by the same genotype. Longevity and fat in this study was unfavourably affected by genotype 2200 (GgB 10). Other genotypes that affected longevity unfavourably were 1010 (GgB 1) and 1100 (GgB 2). While 1101 (GgB 3) affected FCR and EMA favourably, it affected marbling unfavourably. Genotype 2101 (GgB 7) had the most positive and favourable effect on WW_{DIR}, FCR and EMA but affected BW_{DIR} unfavourably. Fat and marbling in this study were affected by genotype 2201 (GgB 11) unfavourably.

In the Drakensberger cattle, the genotype 111 (GgD 03) had a negative and unfavourable effect on SC. Genotype 101 (GgD 01) showed a positive and favourable trend on WW_{DIR}, ADG and FCR. There is a lack of evidence in the literature to verify our findings. These results clearly show that there was an additive effect when more than one variant is present in the *MSTN* gene which affected traits in both SA Bonsmara and Drakensberger cattle. The genotypic combinations in this study could possibly be used as markers to assist breeders to select for desirable phenotypes in breeding programs. Studies have demonstrated the value of effective molecular markers used in selection programs for enhancing traits of economic importance (Zhao *et al.*, 2020).

5.5 Conclusion

The results of this study improve the current understanding of the variability of the *MSTN* gene variants and identified those that have a positive effect (earlier AFC or shorter ICP) or negative impact (decreased marbling) in SA beef breeds. In addition, the combined genotypes revealed that there is an additive effect on economically importance traits when more than one variant are present in the *MSTN* gene. This will assist breeders to select for or against specific *MSTN* variants in their breeding programs to improve production. The advances in modern technology to genotype a larger number of animals will help cattle breeders to identify *MSTN* variants within their herds and develop a breeding strategy to maximize its potential and to achieve their breeding objective.

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